SUPPORTING INFORMATION

Total Synthesis of Polysaccharides by Automated Glycan Assembly

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General Materials and Methods

All chemicals used were reagent grade and used as supplied unless otherwise noted. All building blocks used were purchased from *GlycoUniverse*, Germany. Automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces.[1] Merrifield resin LL (100-200 mesh, Novabiochem^{TM}) was modified and used as solid support.[2] Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a p-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04 – 0.063 mm). Analysis and purification by normal and reverse phase HPLC was performed using an Agilent 1200 series. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. ^{1}H, ^{13}C and HSQC NMR spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in $CDCl_3$ by using the solvent residual peak chemical shift as the internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or in D_2O using the solvent as the internal standard in ^{1}H NMR (D_2O: 4.79 ppm ^{1}H) and a D6-acetone spike as the internal standard in ^{13}C NMR (acetone in D_2O: 30.89 ppm ^{13}C) unless otherwise stated. High resolution mass spectra were obtained using a 6210 ESI-TOF mass spectrometer (Agilent) and a MALDI-TOF Autoflex^{TM} (Bruker). MALDI and ESI mass spectra were run on IonSpec Ultima instruments.

Solvents used for dissolving building block and preparing the activator, TMSOTf and capping solutions were taken from an anhydrous solvent system (jcmeyer-solvent systems). Other solvents used were HPLC grade. The building blocks were co-evaporated three times with toluene and dried 2 h under high vacuum before use. Activator, deprotection, acidic wash, capping and building block solutions were freshly prepared and kept under argon during the automation run. All yields of products obtained by AGA were calculated based on resin loading. Resin loading was determined by performing one glycosylation (Module C) with ten equivalents of building block followed by DBU promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance.

Preparation of Stock Solutions [3]

- **Building block:** building block was dissolved in 1 mL dichloromethane (DCM).
- **Activator solution:** Recrystallized NIS (1.56 g) was dissolved in 60 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then triflic acid (67 µL) was added. The solution is kept at 0°C for the duration of the automation run.
- **Fmoc deprotection solution:** A solution of 20% piperidine in dimethylformamide (DMF) (v/v) was prepared.
- **TMSOTf solution:** Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.9 mL) was added to DCM (90 mL).
- **Capping solution:** A solution of 10% acetic anhydride (Ac_2O) and 2% methanesulfunic acid (MsOH) in anhydrous DCM (v/v) was prepared.
- **Lev deprotection solution**: Hydrazine acetate (550 mg) was dissolved in a solution of 4:1:0.25 pyridine:AcOH:H_2O (40 mL).

Modules for Automated Synthesis

Module A: Resin Preparation for Synthesis (20 min)

All automated syntheses were performed on 19 µmol scale (60 mg). Resin was placed in the reaction vessel and swollen in DCM for 20 min at room temperature prior to synthesis. During this time, all reagent lines required for the synthesis were washed and primed. Before the first glycosylation, the resin was washed with the DMF, tetrahydrofuran (THF), and DCM (three times each with 2 mL for 25 s). This step is conducted as the first step for every synthesis.

Module B: Acidic Wash with TMSOTf Solution (20 min)

The resin was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -20 °C. Upon reaching the temperature, TMSOTf solution (1 mL) was added drop wise to the reaction vessel. After bubbling for argon 3 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

Module C: Thioglycoside Glycosylation (20-60 min)

The building block solution (0.095-0.123 mmol (5-6.5 equivalents) of BB in 1 mL of DCM per glycosylation) was delivered to the reaction vessel. After the set temperature (-20 ^{o}C) was reached, the reaction was started by drop wise addition of the activator solution (1.0 mL, excess). The glycosylation was performed by increasing the temperature to 0 ^{o}C for 20-60 min (depending on oligosaccharide length). After completion of the reaction, the solution is drained and the resin was washed with DCM, DCM:dioxane (1:2, 3 mL for 20 s) and DCM (twice, each with 2 mL for 25 s). The temperature of the reaction vessel is increased to 25 °C for the next module.

Module D: Capping (30 min)

The resin was washed with DMF (twice with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. Pyridine solution (2 mL, 10% in DMF) was delivered into the reaction vessel. After 1 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s). The capping solution (4 mL) was delivered into the reaction vessel. After 20 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s).

Module E: Fmoc Deprotection (14 min)

The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. Fmoc deprotection solution (2 mL) was delivered into the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 3 mL for 25 s) and DCM (five times each with 2 mL for 25 s). The temperature of the reaction vessel is decreased to -20 °C for the next module.

Module F: Lev Deprotection (ca. 100 min)

The resin was washed with DMF (3×30 sec) and DCM (1.3 mL) added to the reaction vessel. Solution **F** (0.8 mL) was added to the reaction vessel, and the temperature was adjusted to 25 °C. After 30 min, the reaction solution was drained and the entire cycle was repeated twice more. After Lev deprotection was complete, the resin was washed with DMF, THF and DCM.

Post-Synthesizer Manipulations

Cleavage from Solid Support

After automated synthesis, the oligosaccharides were cleaved from the solid support using a continuous-flow photo reactor as described previously.[4]

Purification

Solvent was evaporated *in vacuo* and the crude products were dissolved in a 1:1 mixture of hexane and ethyl acetate and analyzed using analytical HPLC (DAD1F, 280 nm). Pure compounds were afforded by preparative HPLC (Agilent 1200 Series spectrometer).

Method A: (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 50% EtOAc as eluents [isocratic 50% EtOAc (5 min), linear gradient to 80% EtOAc (5 min), linear gradient to 75% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)].

Method B: (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 50% EtOAc as eluents [isocratic 50% EtOAc (5 min), linear gradient to 80% EtOAc (75 min), linear gradient to 100% EtOAc (10 min)].

Method C: (Synergi Hydro RP18 column, 250 x 4.6 mm) flow rate of 1.0 mL / min with water (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 10% ACN (30 min), linear gradient to 100% ACN (5 min)].

Method D: (Synergi Hydro RP18 column, 250 x 10 mm) flow rate of 4.0 mL / min with water (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 10% ACN (30 min), linear gradient to 100% ACN (5 min)].

Method E: (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 35% EtOAc as eluents [isocratic 35% EtOAc (5 min), linear gradient to 60% EtOAc (5 min), linear gradient to 60% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)].

Method F: (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 35% EtOAc as eluents [isocratic 35% EtOAc (5 min), linear gradient to 60% EtOAc (75 min), linear gradient to 100% EtOAc (10 min)].

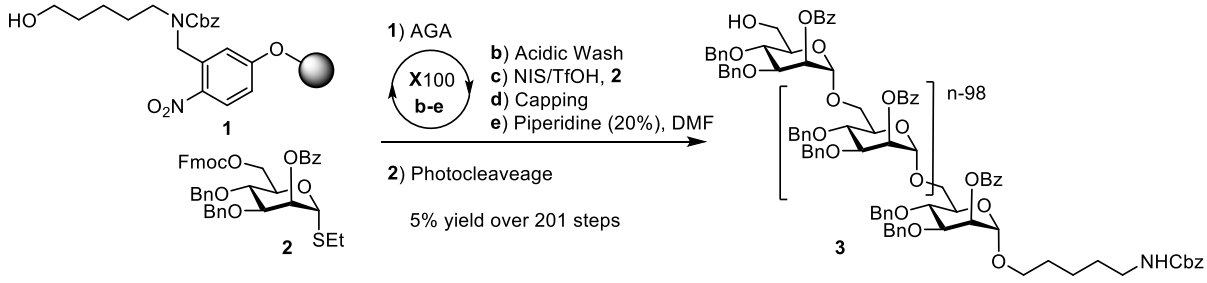
Method G: (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 40% EtOAc as eluents [isocratic 40% EtOAc (5 min), linear gradient to 70% EtOAc (5 min), linear gradient to 70% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)].

Method H: (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 40% EtOAc as eluents [isocratic 40% EtOAc (5 min), linear gradient to 70% EtOAc (75 min), linear gradient to 100% EtOAc (10 min)].

Method I: (TSKgel G 3000 PWXL column, 7.8 mm ID x 30 cm) flow rate of 0.4 mL / min with water as an eluent.

Synthesis of 100-mer Polymannoside 4

AGA of 100-mer polymannoside **3**

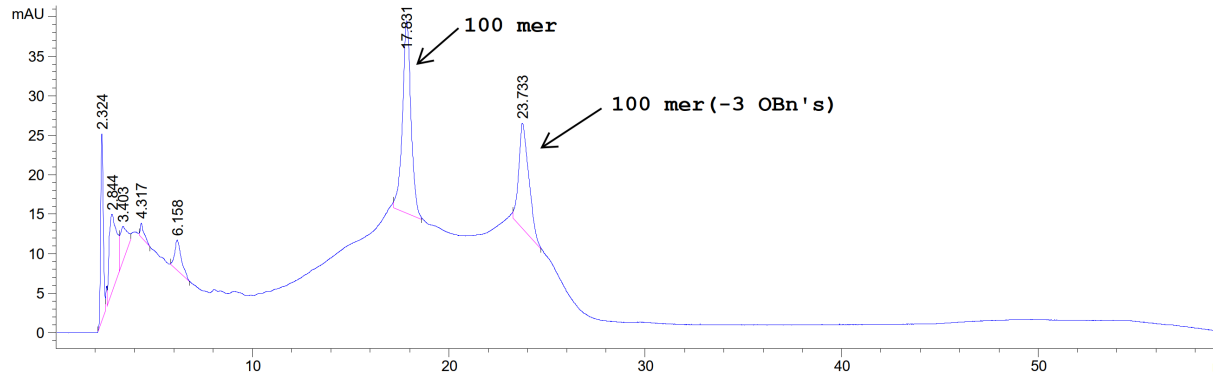


Module	Conditions	Cycles
A: Resin Preparation for Synthesis		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (5 equiv)	
	-20° for 5 min, 0° for 20 min	40
D: Capping		
E: Fmoc Deprotection		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (6 equiv)	
	-20° for 5 min, 0° for 40 min	40
D: Capping		
E: Fmoc Deprotection		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (6.5 equiv)	
	-20° for 5 min, 0° for 60 min	20
D: Capping		
E: Fmoc Deprotection		

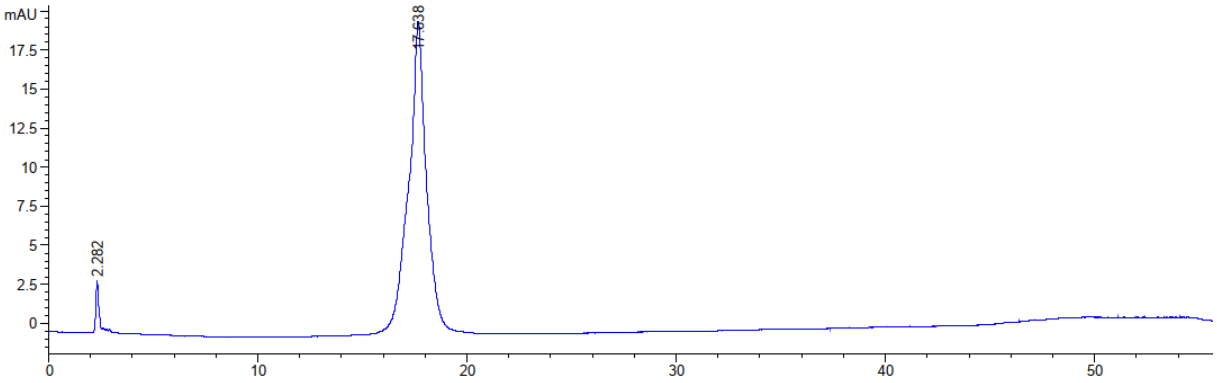
The product was cleaved from the solid support as described in the post-synthesizer manipulations followed by purification using normal phase preparative HPLC with a YMC diol column (Method B) Linear gradient: Hex – 50% EtOAc as eluents [isocratic 50% EtOAc (5 min),

linear gradient to 50% EtOAc (5 min), linear gradient to 80% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)] 100 mer **3**, **S1**(-3 OBn's) eluted at 17.63, 24.42 minutes respectively.

Analytical HPLC (Method A, 280 nm) of crude protected 100-mer (**3**)

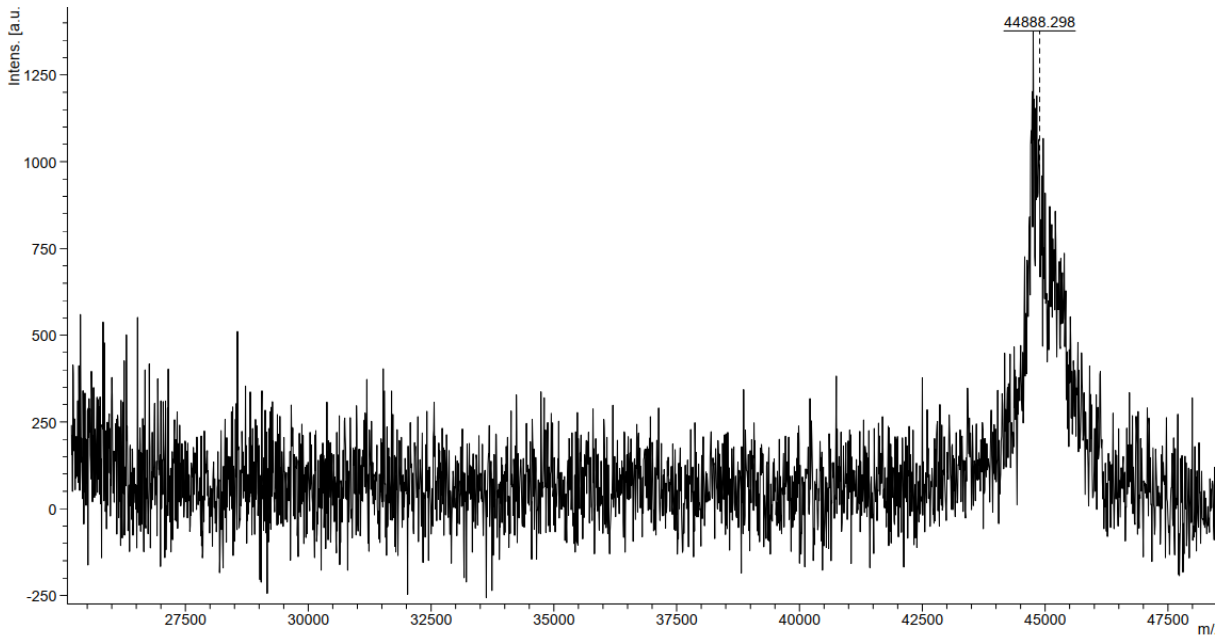


Analytical HPLC (Method A, 280 nm) of pure protected 100-mer (**3**)

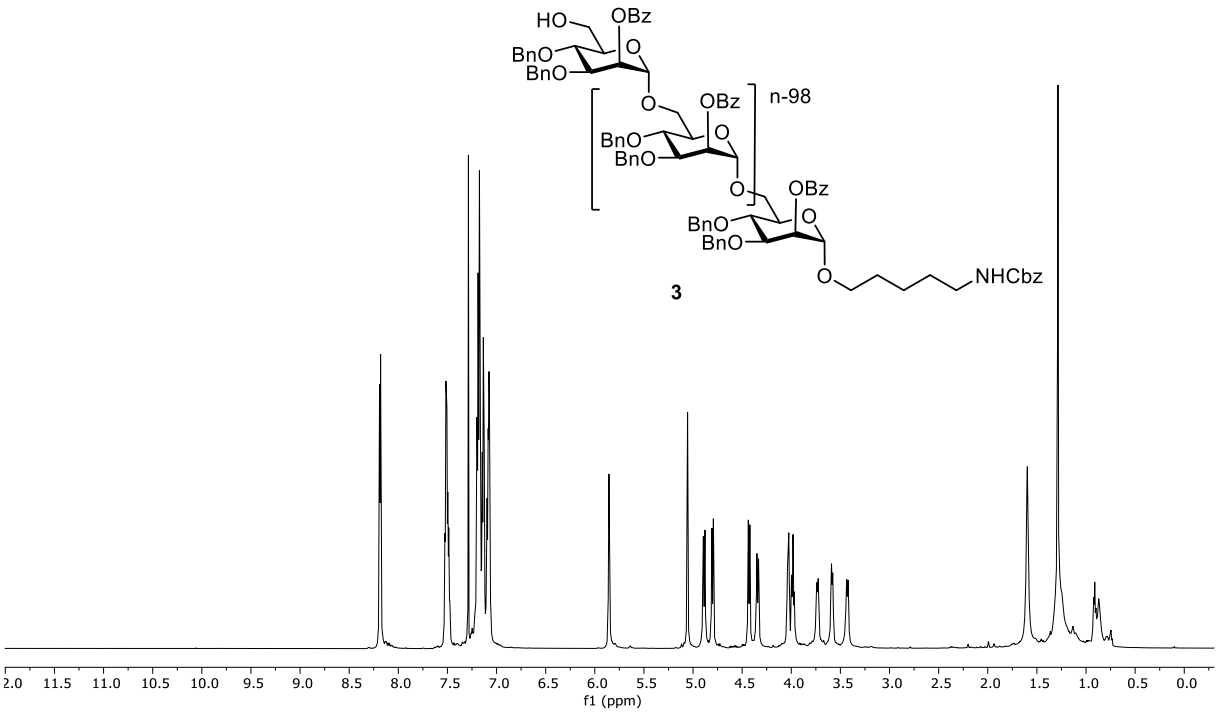


Analytical data for 100-mer polymannose **3**: Yield 5% over 201 steps. ^{1}H NMR (700 MHz, Chloroform-*d*) δ 8.16 (d, *J* = 7.3 Hz, 200H), 7.48 (dt, *J* = 13.6, 7.2 Hz, 250H), 7.21 – 6.99 (m, 1060H), 5.83 (s, 100H), 5.03 (s, 100H), 4.86 (d, *J* = 11.5 Hz, 100H), 4.78 (d, *J* = 10.8 Hz, 100H), 4.40 (d, *J* = 10.8 Hz, 100H), 4.31 (d, *J* = 11.7 Hz, 100H), 4.04 – 3.98 (m, 100H), 3.96 (t, *J* = 9.5 Hz, 100H), 3.70 (d, *J* = 10.6 Hz, 100H), 3.56 (d, *J* = 9.5 Hz, 1H), 3.40 (d, *J* = 11.0 Hz, 100H), 3.16 – 3.15 (m, 2H), 1.58 – 1.53 (m, 2H), 1.49 (m, 2H), 1.36 – 1.32 (m, 2H); ^{13}C NMR (176 MHz, Chloroform-*d*) δ 165.54, 138.49, 137.52, 133.32, 130.02, 129.87, 128.66, 128.37, 128.34, 128.14, 127.69, 127.30, 127.01, 98.56, 78.21, 75.00, 73.72, 71.30, 70.90, 68.39, 65.73, 29.12; m/z (MALDI-TOF) 44888.298 $[M + Na]^+$ ($C_{2713}H_{2619}NO_{603}Na$ requires 44887.4201).

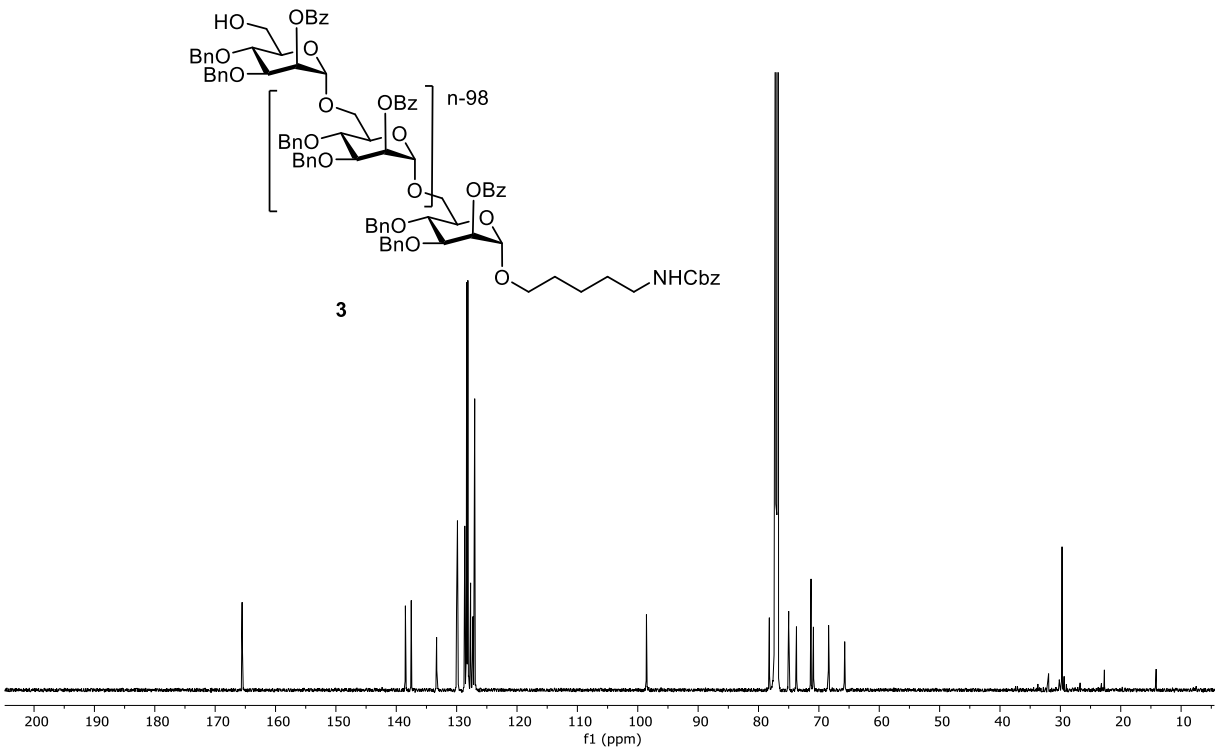
MALDI-TOF of protected 100-mer (**3**)



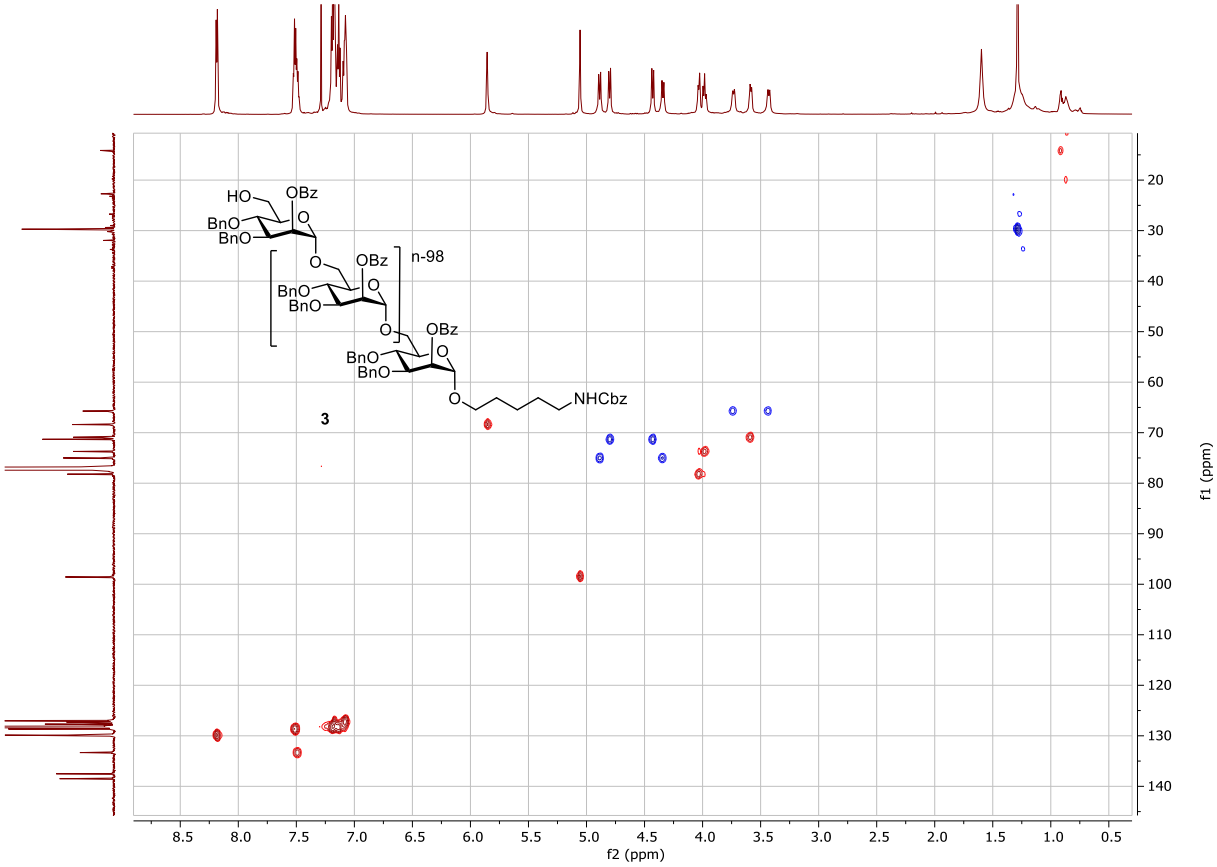
^{1}H NMR of protected 100-mer (**3**)



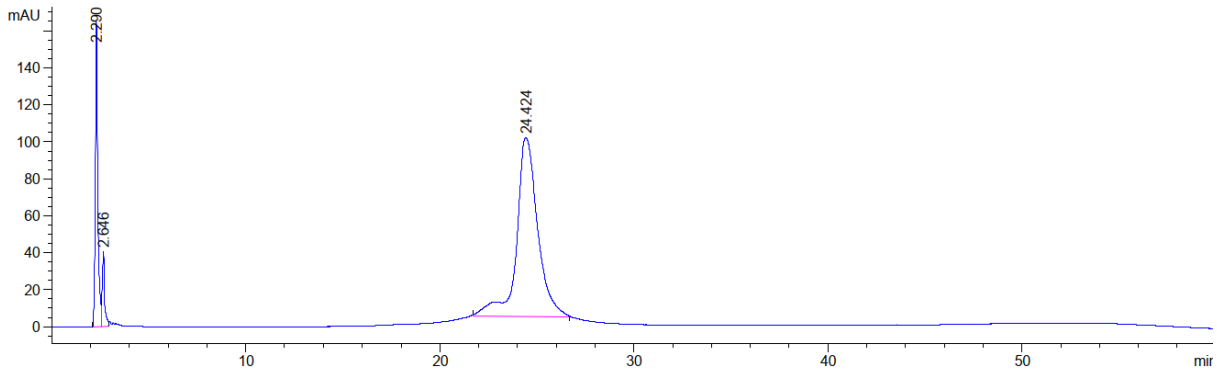
^{13}C NMR of protected 100-mer (**3**)



HSQC NMR of protected 100-mer (**3**)

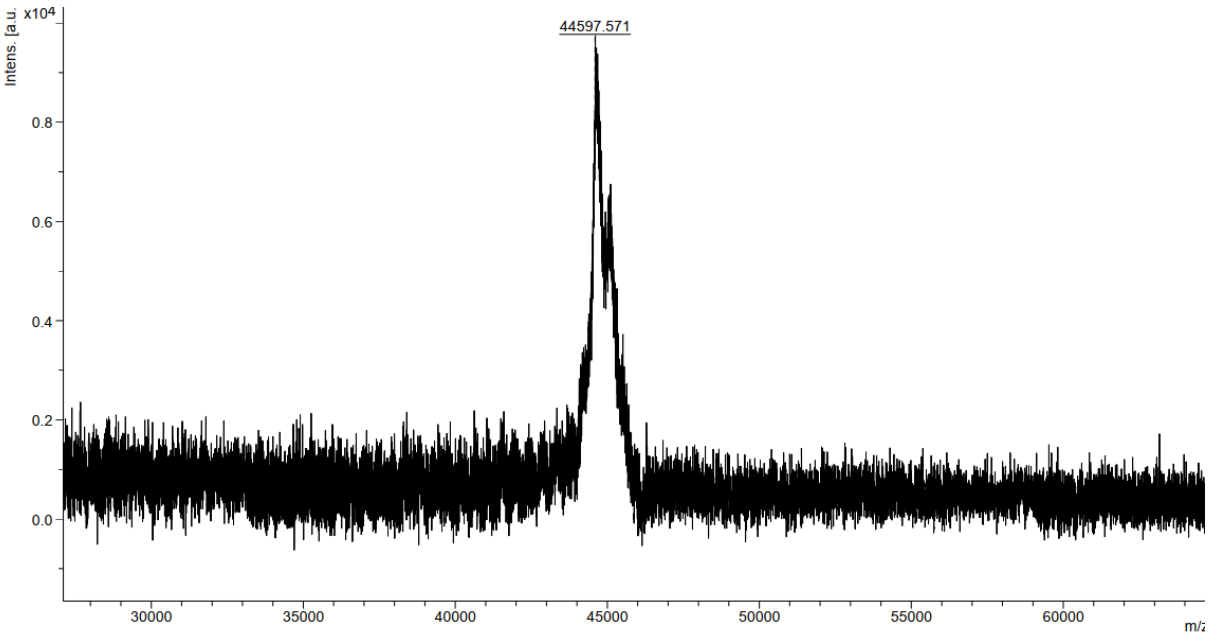


Analytical HPLC (Method A, 280 nm) of pure protected 100-mer (**S1**)

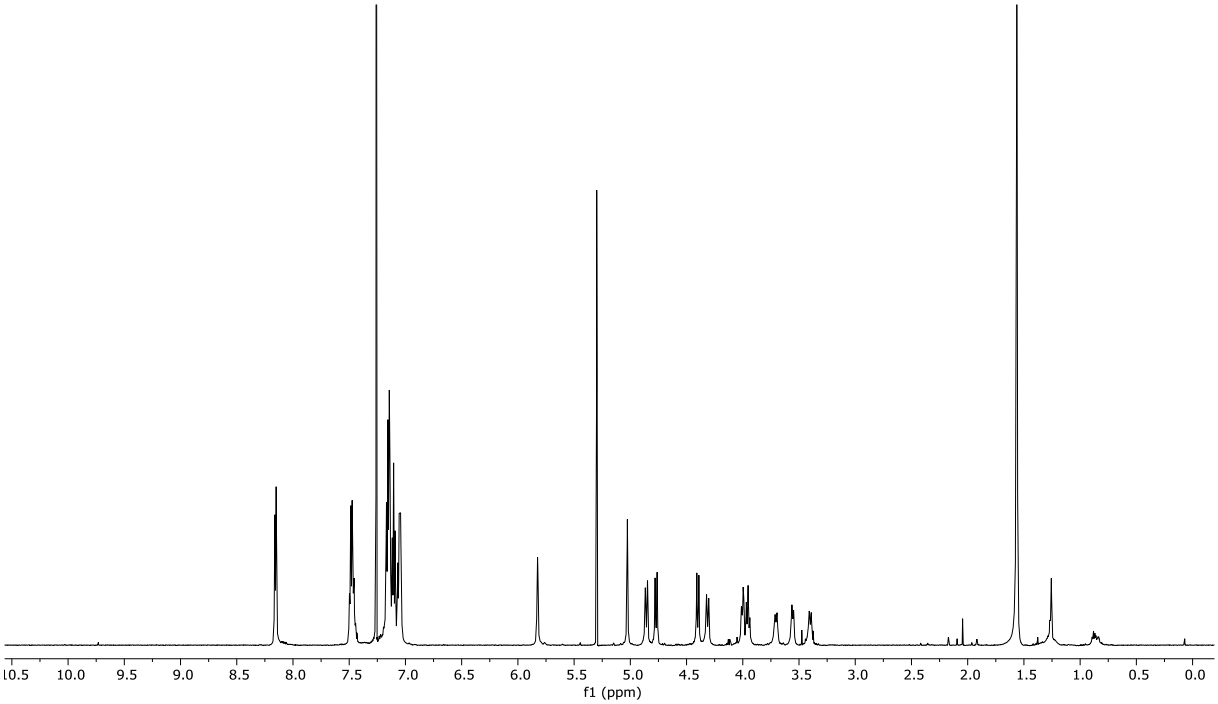


Analytical data for 100-mer polymannose **S1**: Yield 3% over 201 steps. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.18 – 8.13 (m, 200H), 7.52 – 7.44 (m, 250H), 7.19 – 7.05 (m, 1045H), 5.82 (s, 100H), 5.03 (d, *J* = 1.7 Hz, 100H), 4.86 (d, *J* = 11.6 Hz, 100H), 4.77 (d, *J* = 10.8 Hz, 100H), 4.40 (d, *J* = 10.8 Hz, 100H), 4.31 (d, *J* = 11.7 Hz, 100H), 4.03 – 3.93 (m, 200H), 3.70 (d, *J* = 10.8 Hz, 100H), 3.56 (d, *J* = 9.6 Hz, 100H), 3.40 (q, *J* = 9.5 Hz, 100H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 165.68, 138.62, 137.64, 133.46, 130.14, 130.00, 128.79, 128.51, 128.48, 128.28, 127.83, 127.43, 127.14, 98.69, 78.34, 75.13, 73.84, 71.43, 71.02, 68.52, 65.85; m/z (MALDI-TOF) 44597.5710 $[M + Na]^+$ ($C_{2692}H_{2601}NO_{603}Na$ requires 44607.2793).

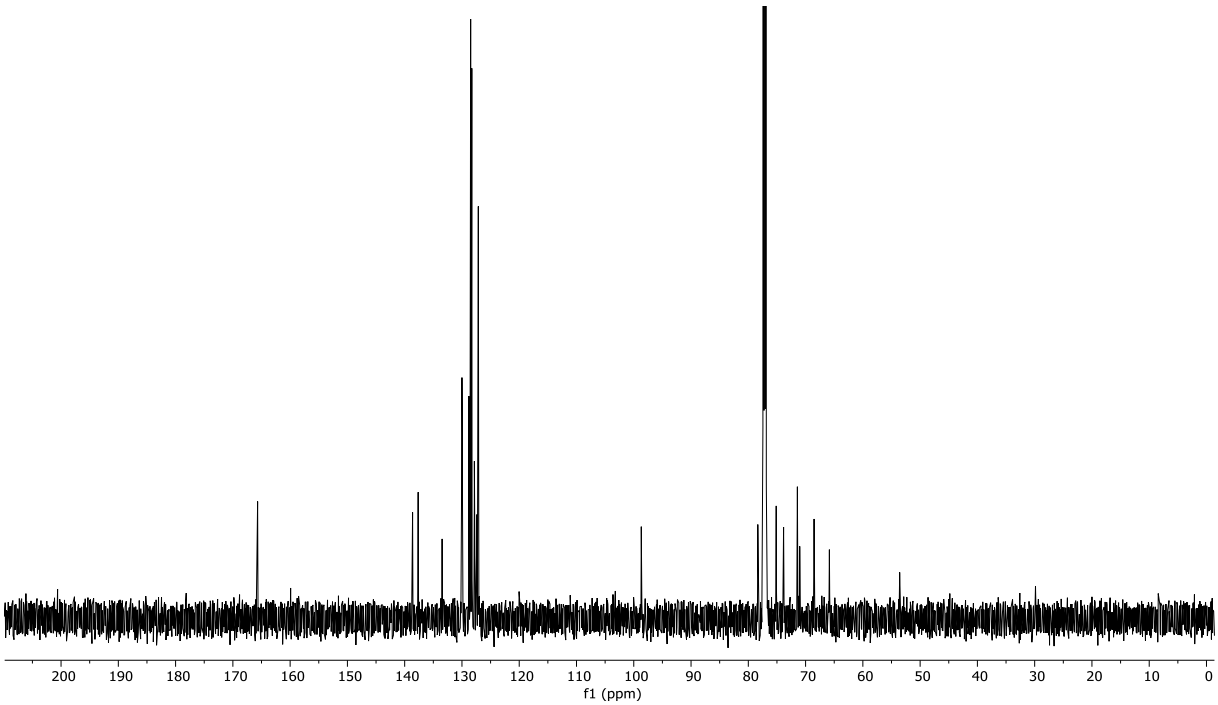
MALDI-TOF of protected 100-mer (**S1**)



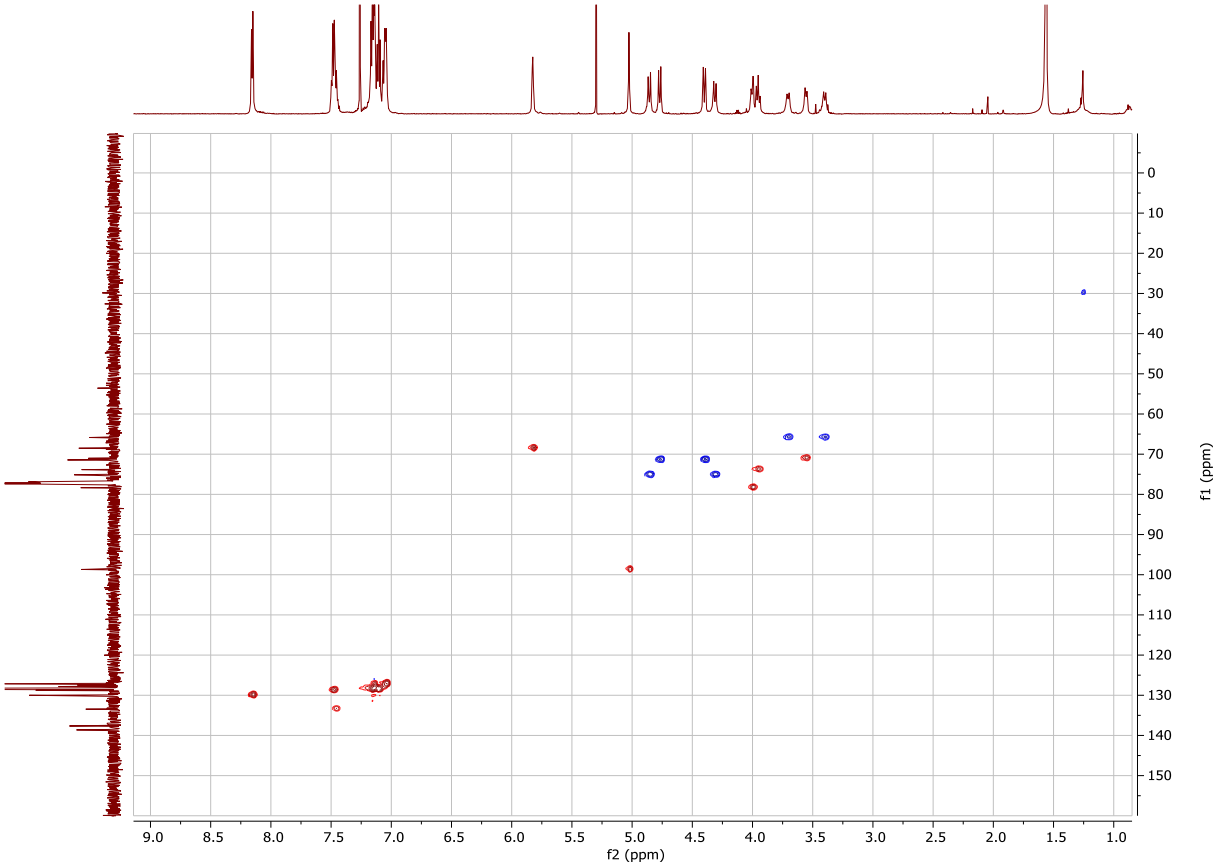
^{1}H NMR of protected 100-mer (**S1**)



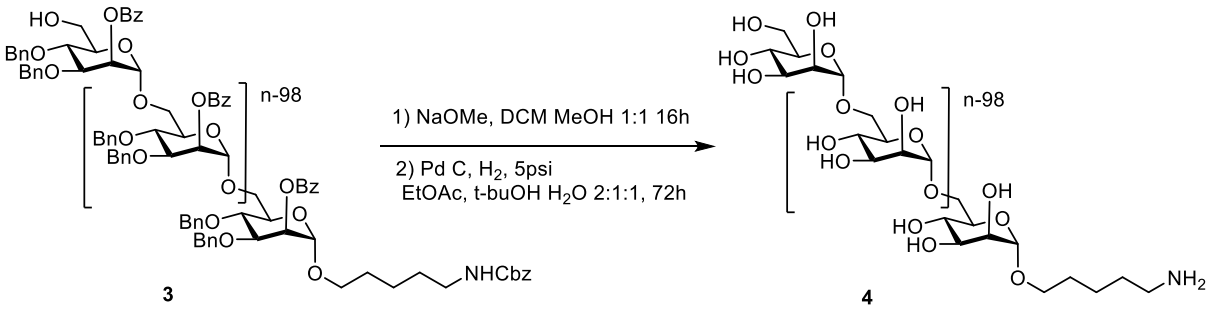
^{13}C NMR of protected 100-mer (**S1**)



HSQC NMR of protected 100-mer (**S1**)



Deprotection of 100-mer Polymannoside:

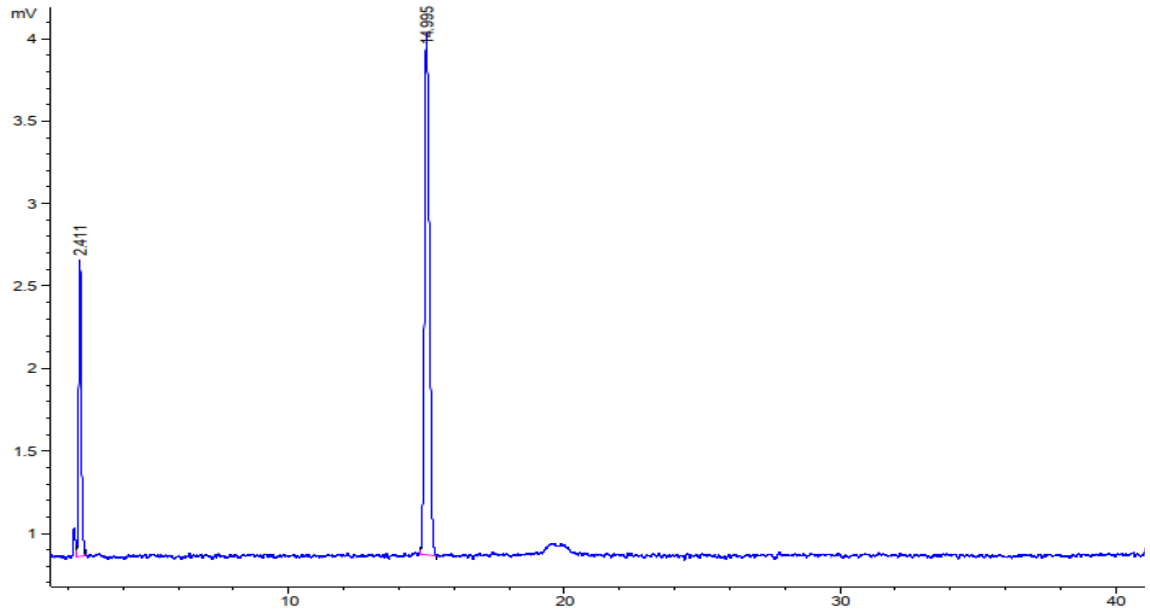


Zemplén Methanolysis. To a solution of protected oligosaccharide **3** in methanol:CH_2Cl_2 (1:1), was added sodium methoxide in methanol (0.5 M, pH 13) and stirred at room temperature for 16 h, neutralized with Amberlite ion exchange (H+) resin, filtered and concentrated in vacuo and carried forward directly into hydrogenolysis without purification.

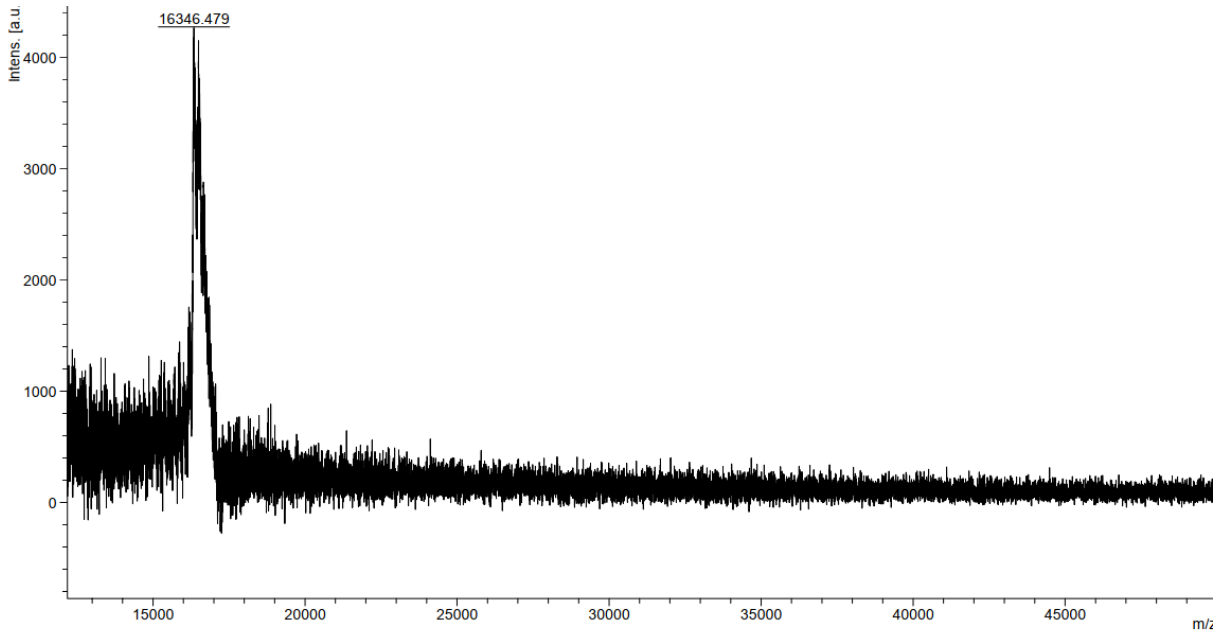
Hydrogenolysis. The product of Zemplén methanolysis was dissolved in EtOAc:*t*-BuOH:H_2O (2:1:1) and transferred to cylindrical vials. Pd-C (10%) (100 weight %) was added and the reaction mixture was stirred under hydrogen reactor with 5 psi pressure for 72 h. The reaction mixture were filtered through Celite and washed with methanol and water. The filtrates were concentrated in vacuo and purified on Hypercarb column (Method D) and lyophilized to give 1 mg (24% yield over two steps) of pure compound **4**.

Analytical data for **4**: ^{1}H NMR (700 MHz, D_2O) δ 4.85 (s, 100H), 3.94 (s, 100H), 3.89 (dd, *J* = 11.4, 5.5 Hz, 100H), 3.84 – 3.76 (m, 200H), 3.73 (d, *J* = 11.1 Hz, 100H), 3.67 (t, *J* = 9.8 Hz, 100H), 3.61 (d, *J* = 10.1 Hz, 2H), 2.95 (d, *J* = 9.6 Hz, 2H), 2.80 (d, *J* = 1.7 Hz, 2H), 1.70 – 1.55 (m, 4H); ^{13}C NMR (176 MHz, Deuterium Oxide) δ 171.06, 99.39, 70.88, 70.74, 70.03, 66.65, 65.57 ; m/z (MALDI-TOF) 16346.479 $[M+K]^+$ ($C_{605}H_{1013}NO_{501}K$ requires 16347.345)

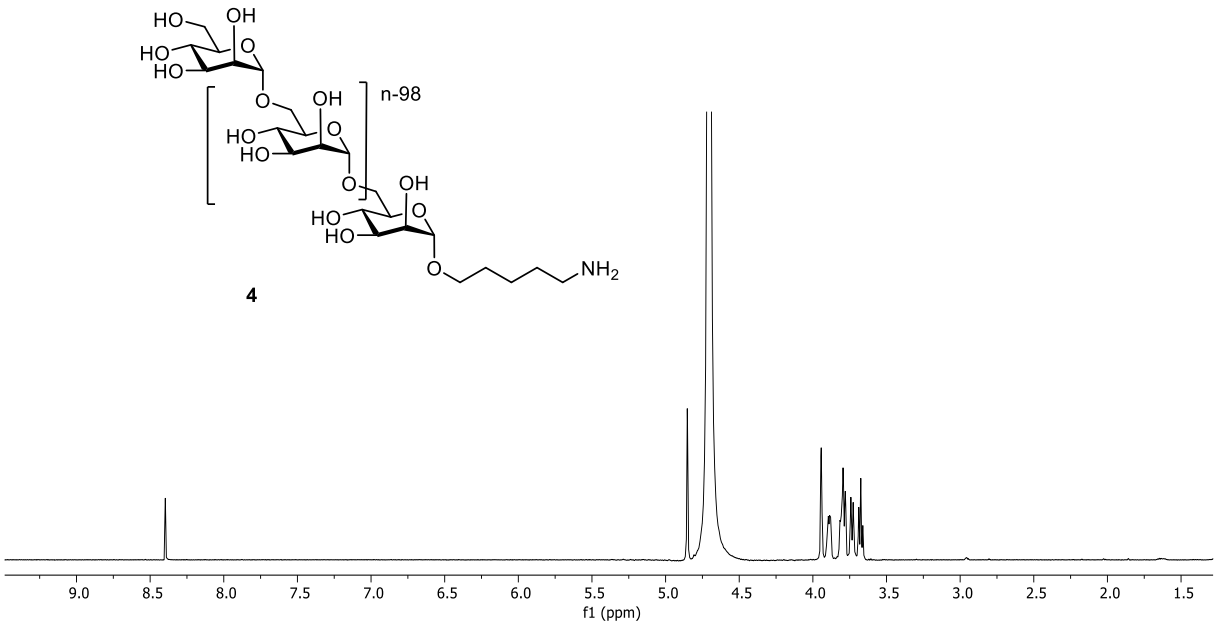
Analytical HPLC (Method C, ELSD trace) of deprotected 100-mer (**4**)



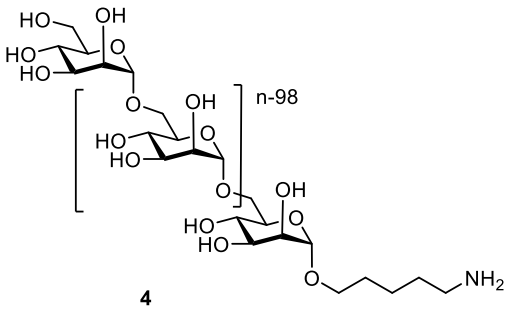
MALDI-TOF spectrum of deprotected 100-mer (**4**)



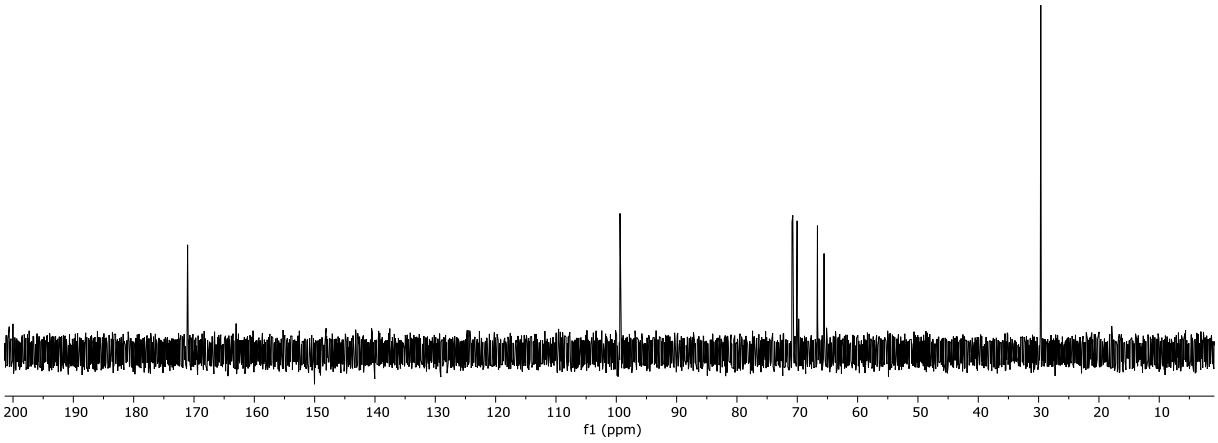
^{1}H NMR of deprotected 100-mer (**4**)



^{13}C NMR of deprotected 100-mer (**4**)

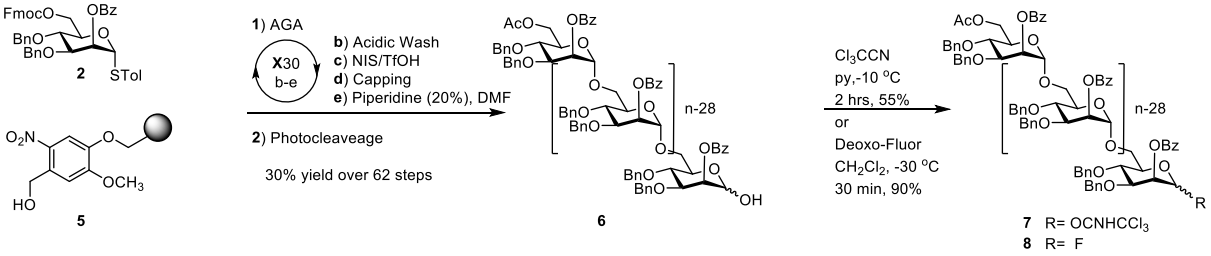


4



Synthesis of 151-mer polymannoside by block coupling:

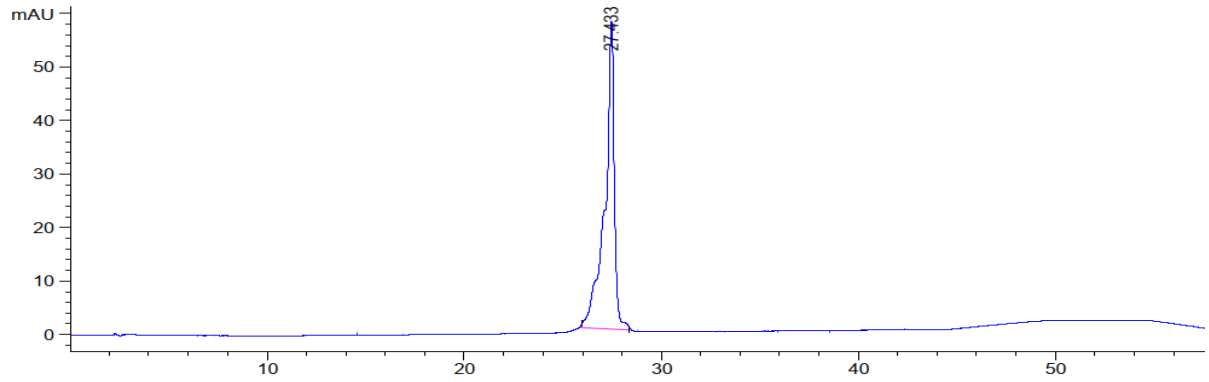
30 mer Donor Synthesis:



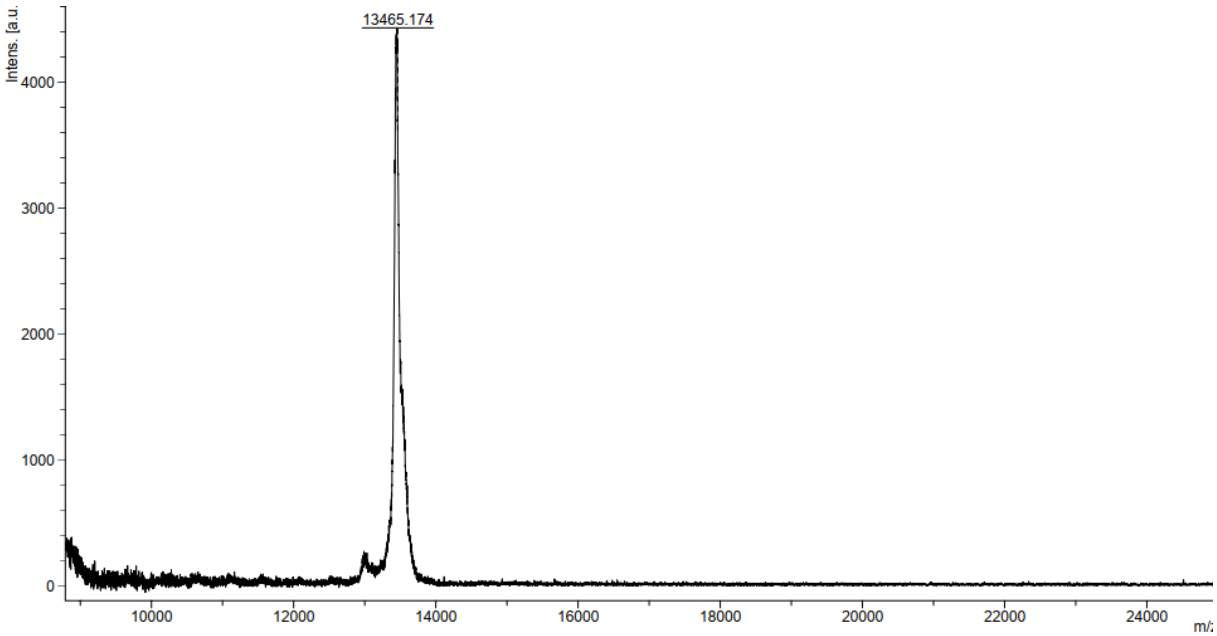
Module	Conditions	Cycles
A: Resin Preparation for Synthesis[2]		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (5 equiv)	
	-20° for 5 min, 0° for 20 min	30
D: Capping		
E: Fmoc Deprotection		
D: Capping		2

The product was cleaved from the solid support as described in the post-synthesizer manipulations followed by purification using normal phase preparative HPLC with a YMC diol column (Method F) Linear gradient: Hex – 35% EtOAc as eluents [isocratic 35% EtOAc (5 min), linear gradient to 35% EtOAc (5 min), linear gradient to 60% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)] 30 mer **6** eluted at 27.43 minutes.

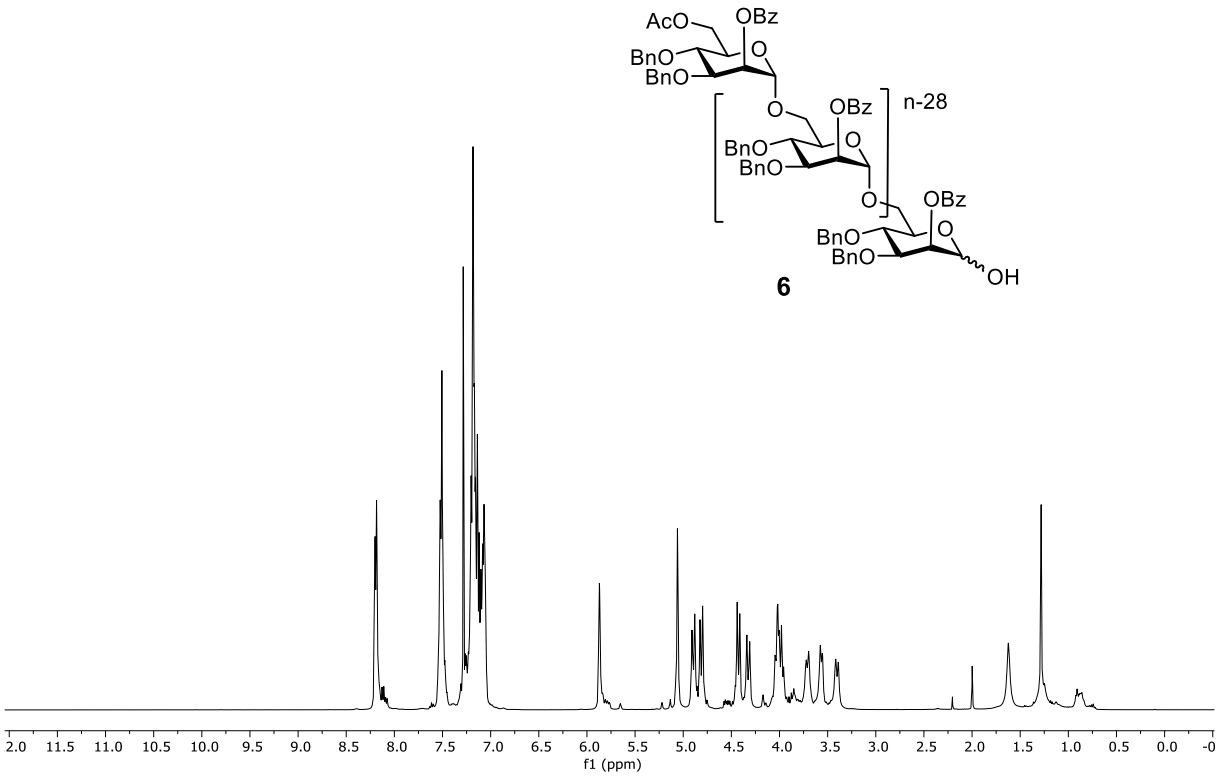
Analytical HPLC (Method E, 280 nm) of 30-mer, 1-OH (**6**)



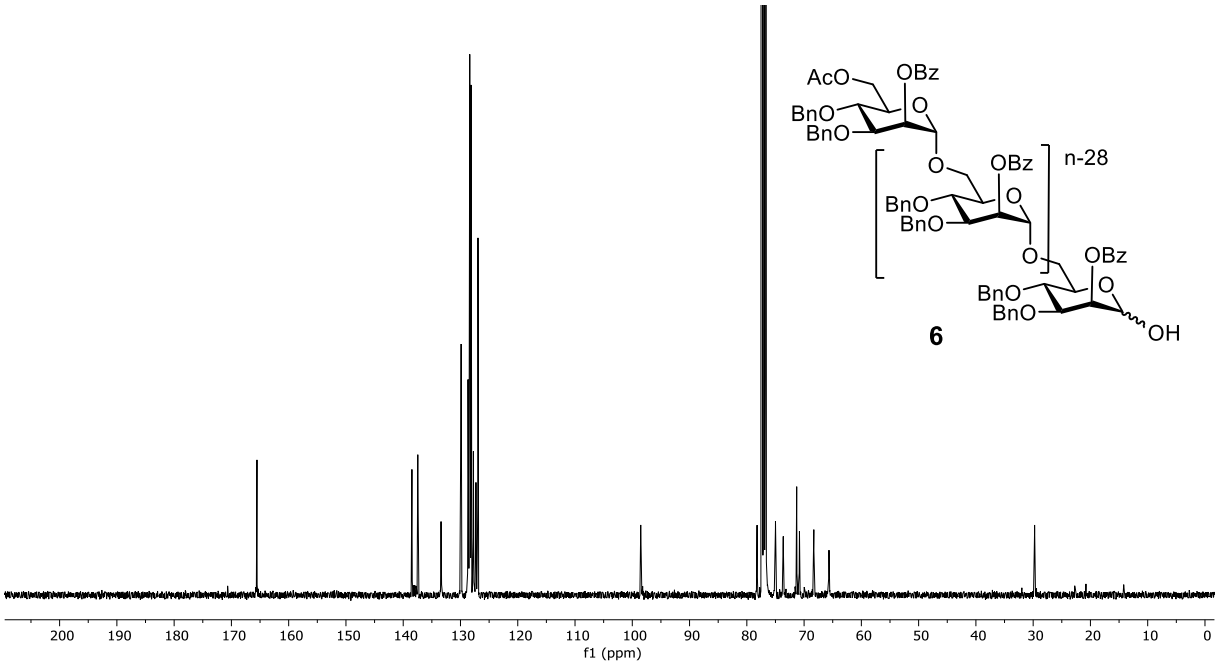
MALDI-TOF spectrum of 30-mer, 1-OH (**6**)



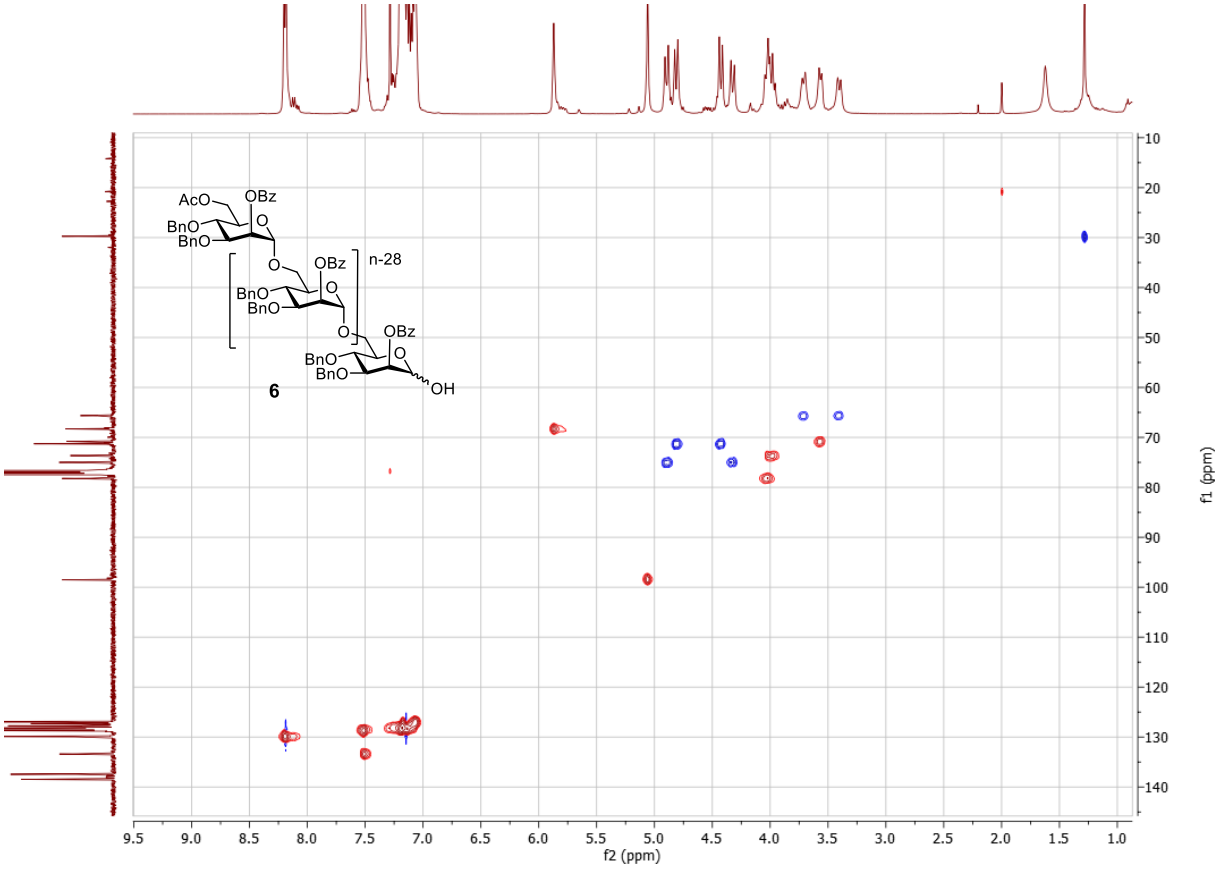
^{1}H NMR of 30-mer, 1-OH (**6**)



^{13}C NMR of 30-mer, 1-OH (**6**)

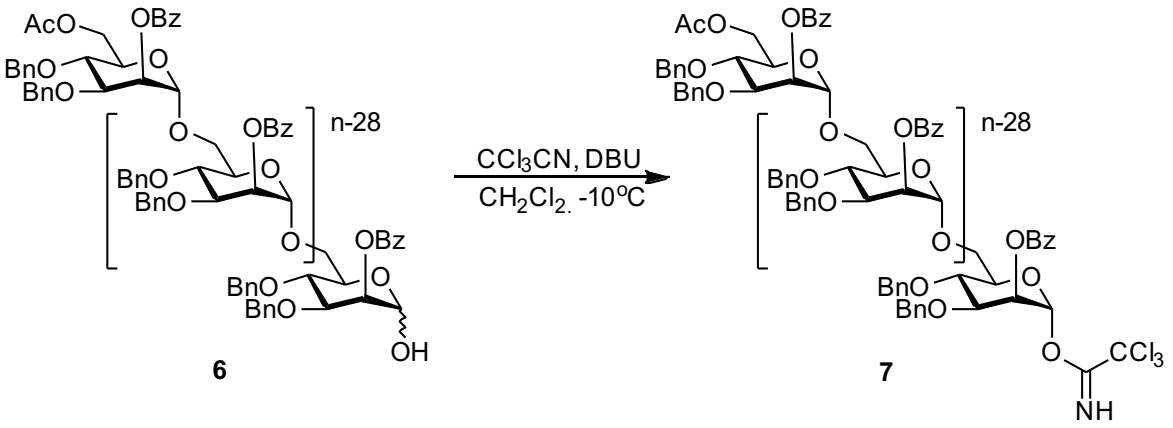


HSQC NMR of 30-mer, 1-OH (**6**)



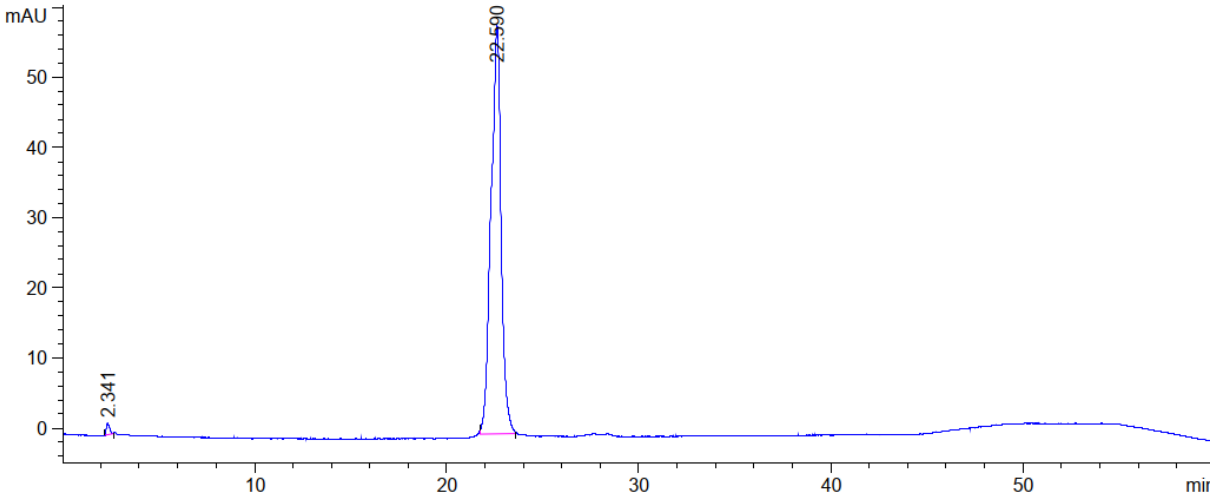
Analytical data for **6**: Yield 76 mg (30% over 62 steps); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 – 8.15 (m, 60H), 7.52 (d, *J* = 7.0 Hz, 120H), 7.24 – 7.03 (m, 360H), 5.87 (t, *J* = 2.3 Hz, 30H), 5.06 (s, 30H), 4.89 (d, *J* = 11.4 Hz, 30H), 4.81 (d, *J* = 10.7 Hz, 30H), 4.43 (d, *J* = 10.7 Hz, 30H), 4.32 (d, *J* = 11.7 Hz, 30H), 4.08 – 3.93 (m, 90H), 3.71 (d, *J* = 10.2 Hz, 30H), 3.57 (d, *J* = 9.3 Hz, 30H), 3.40 (d, *J* = 11.2 Hz, 30H), 2.20 (s, OAc, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.54, 138.48, 137.45, 133.39, 129.96, 129.89, 128.68, 128.44, 128.38, 128.15, 127.74, 127.30, 126.95, 98.52, 78.21, 77.26, 74.99, 73.64, 71.29, 70.81, 68.30, 65.65, 20.78; m/z (MALTI-TOF) 13465.174 $[M + Na]^{+}$ ($C_{812}H_{784}O_{182}Na$ requires 13468.199).

Synthesis of 30-mer glycosyl trichloroacetimidate donor 7:

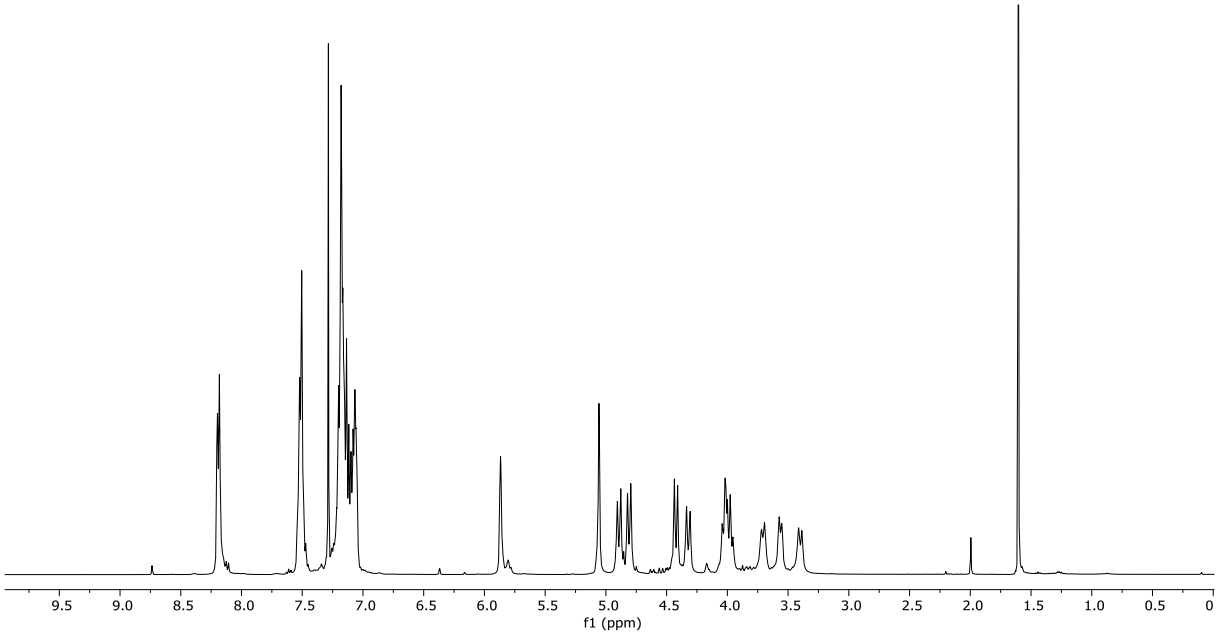


To a solution of compound **6** (60 mg, 4 mmol) in CH_2Cl_2 (2 mL) was added DBU (11 μL, 8 mmol) at -10 °C, to this mixture trichloroacetonitrile (1 mL) was added and allowed to stirred for two hours before the reaction mixture was quenched with water. The organic phase was extracted with $NaHCO_3$ solution, dried over $MgSO_4$, filtered, concentrated and purified by preparative HPLC (Method F) to provide compound **7** (33 mg, 55% yield).

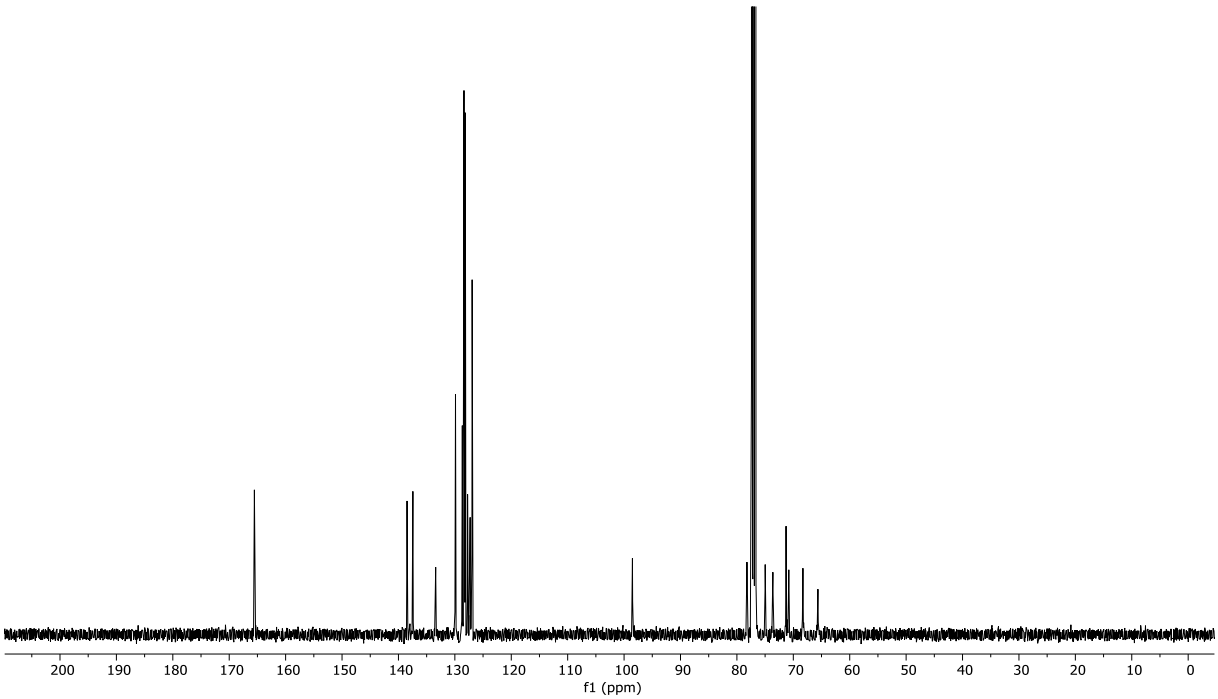
Analytical HPLC (Method E, 280 nm) of 30-mer trichloroacetimidate (**7**)



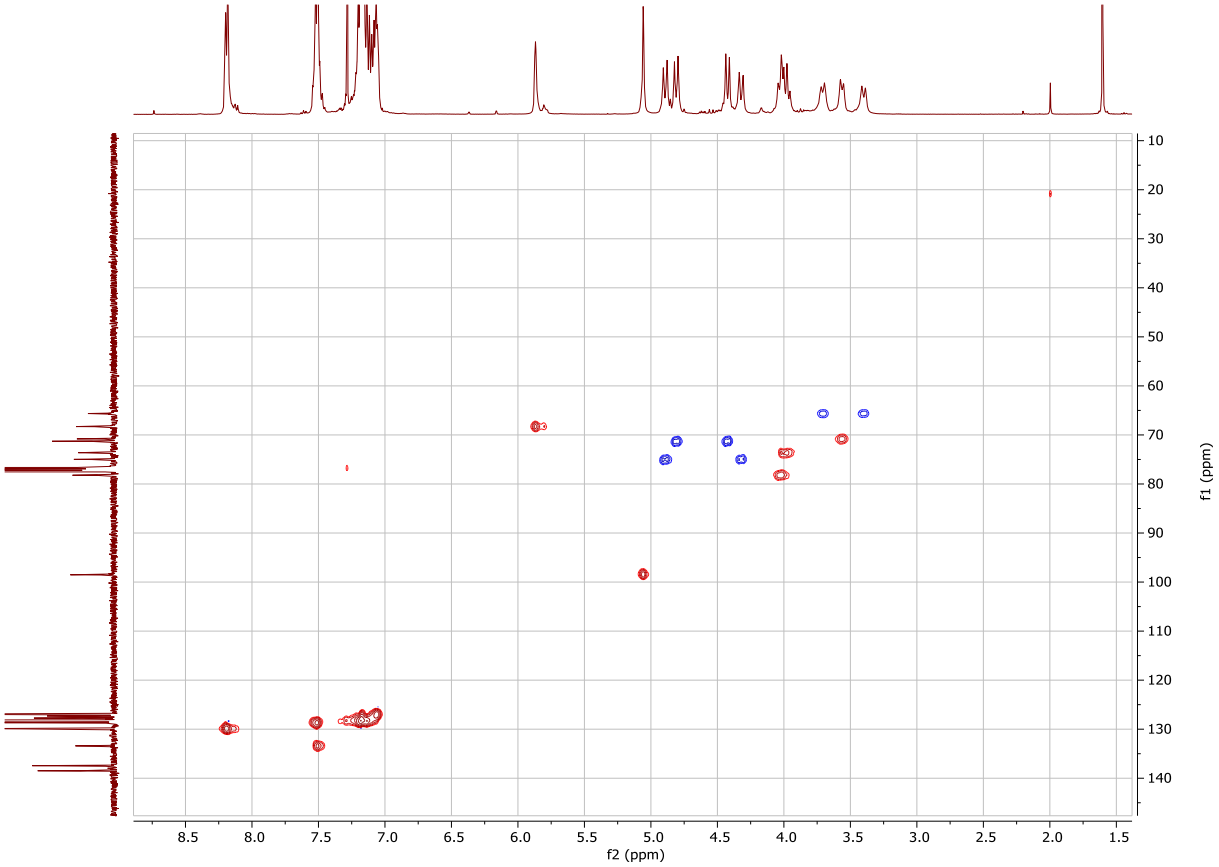
^{1}H NMR of 30-mer trichloroacetimidate (**7**)



^{13}C NMR of 30-mer trichloroacetimidate (**7**)

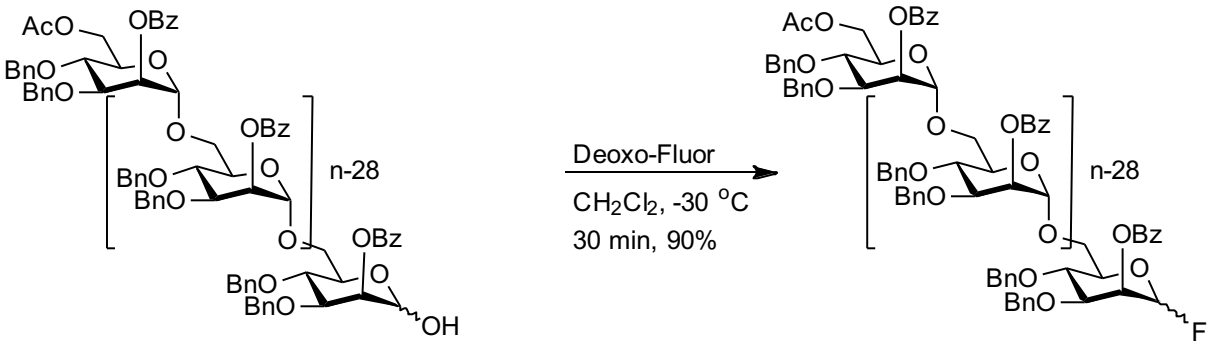


HSQC NMR of 30-mer trichloroacetimidate (**7**)



Analytical data for **7**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 – 8.12 (m, 60H), 7.58 – 7.39 (m, 90H), 7.25 – 7.00 (m, 300H), 5.87 (t, *J* = 2.2 Hz, 30H), 5.06 (d, *J* = 1.7 Hz, 30H), 4.89 (d, *J* = 11.5 Hz, 30H), 4.81 (d, *J* = 11.0 Hz, 30H), 4.42 (d, *J* = 10.8 Hz, 30H), 4.32 (d, *J* = 11.6 Hz, 30H), 4.08 – 3.89 (m, 60H), 3.71 (d, *J* = 10.5 Hz, 30H), 3.56 (d, *J* = 9.4 Hz, 30H), 3.40 (d, *J* = 11.1 Hz, 30H), 2.20 (s, OAc, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.54, 138.48, 137.44, 133.40, 129.95, 129.89, 128.68, 128.44, 128.38, 128.14, 127.75, 127.29, 126.94, 98.52, 78.22, 77.26, 74.99, 73.63, 71.29, 70.80, 68.30, 65.64, 20.74.

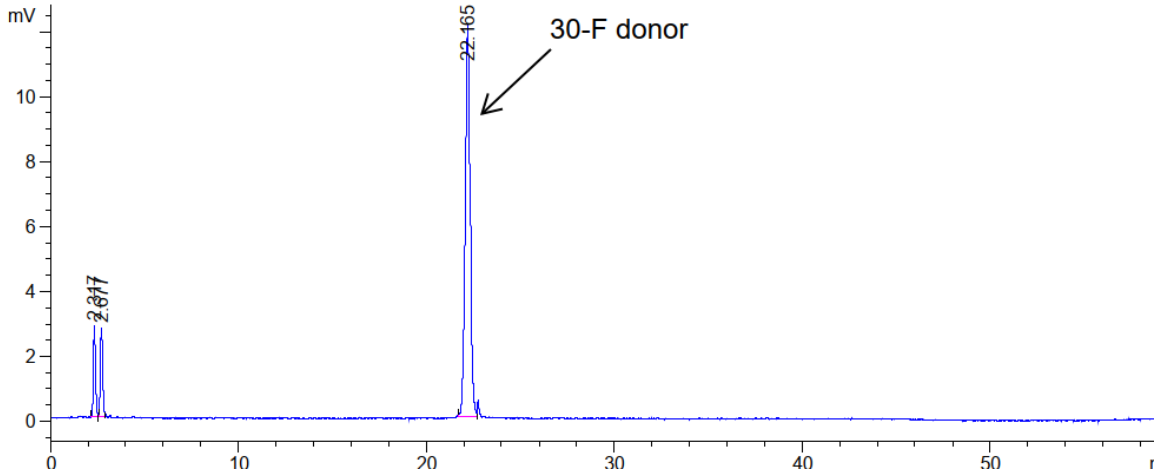
Synthesis of 30 mer glycosyl fluoride donor **8:**



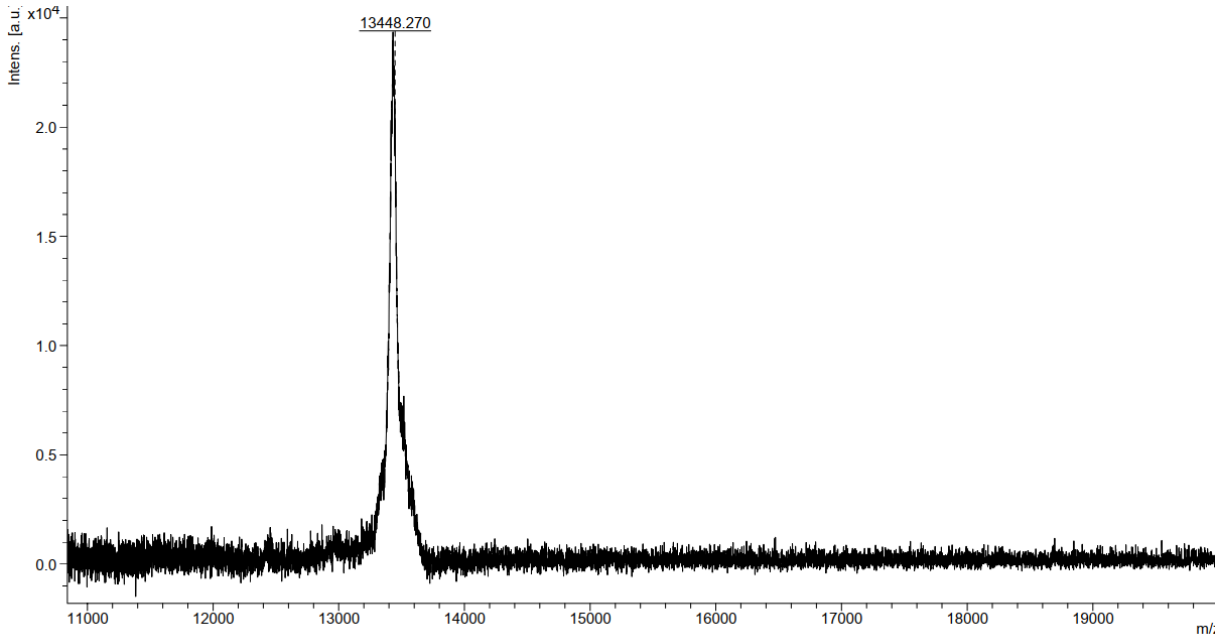
6 **8**

To a solution of compound **6** (94 mg, 72.8 μmol) in CH_2Cl_2 (2 mL) was added a solution of Deoxo-Fluor (110 μL, 150 μmol) at -30 °C. The reaction was stirred for 30 minutes. The reaction was quenched with saturated $NaHCO_3$ solution (2 mL). The organic phase was extracted with aqueous citric acid, dried over $MgSO_4$, filtered, concentrated and purified by preparative HPLC (Method F)to provide compound **8** (85 mg) in 90% yield.

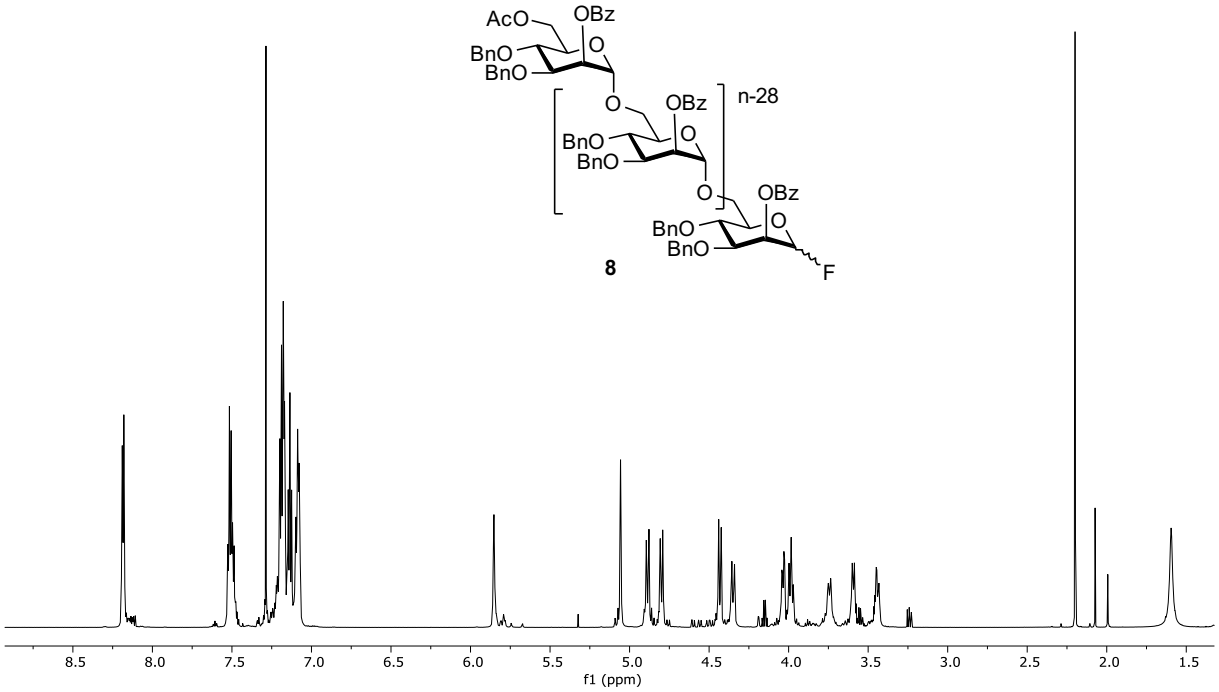
Analytical HPLC (Method E, ELSD) of 30-F donor (**8**)



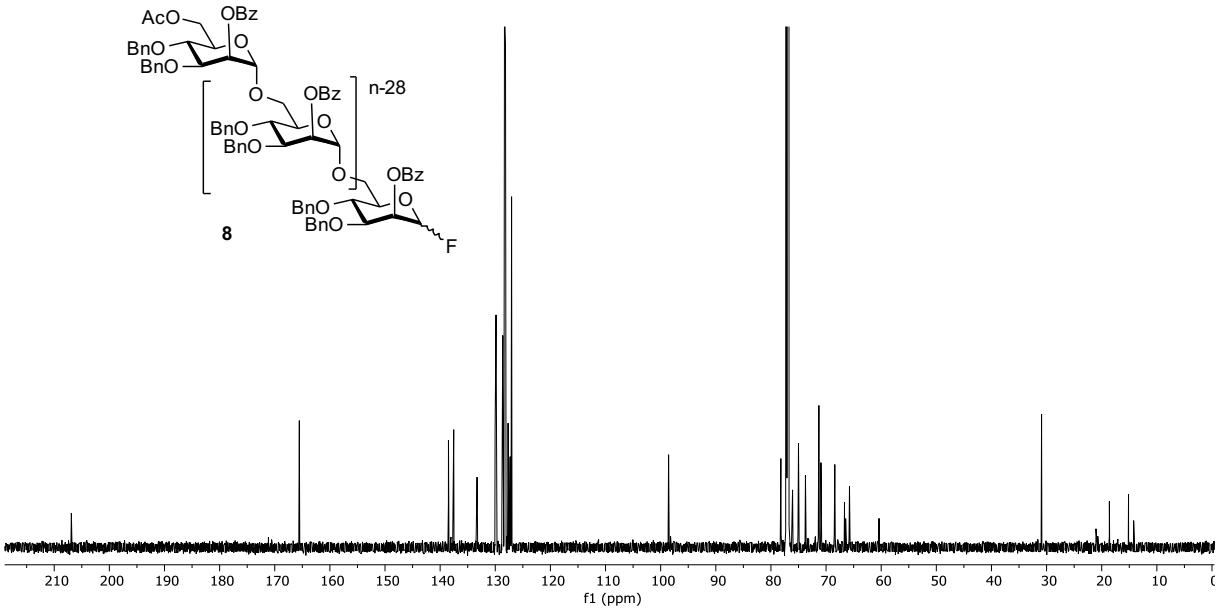
MALDI-TOF spectrum of 30-F donor (**8**)



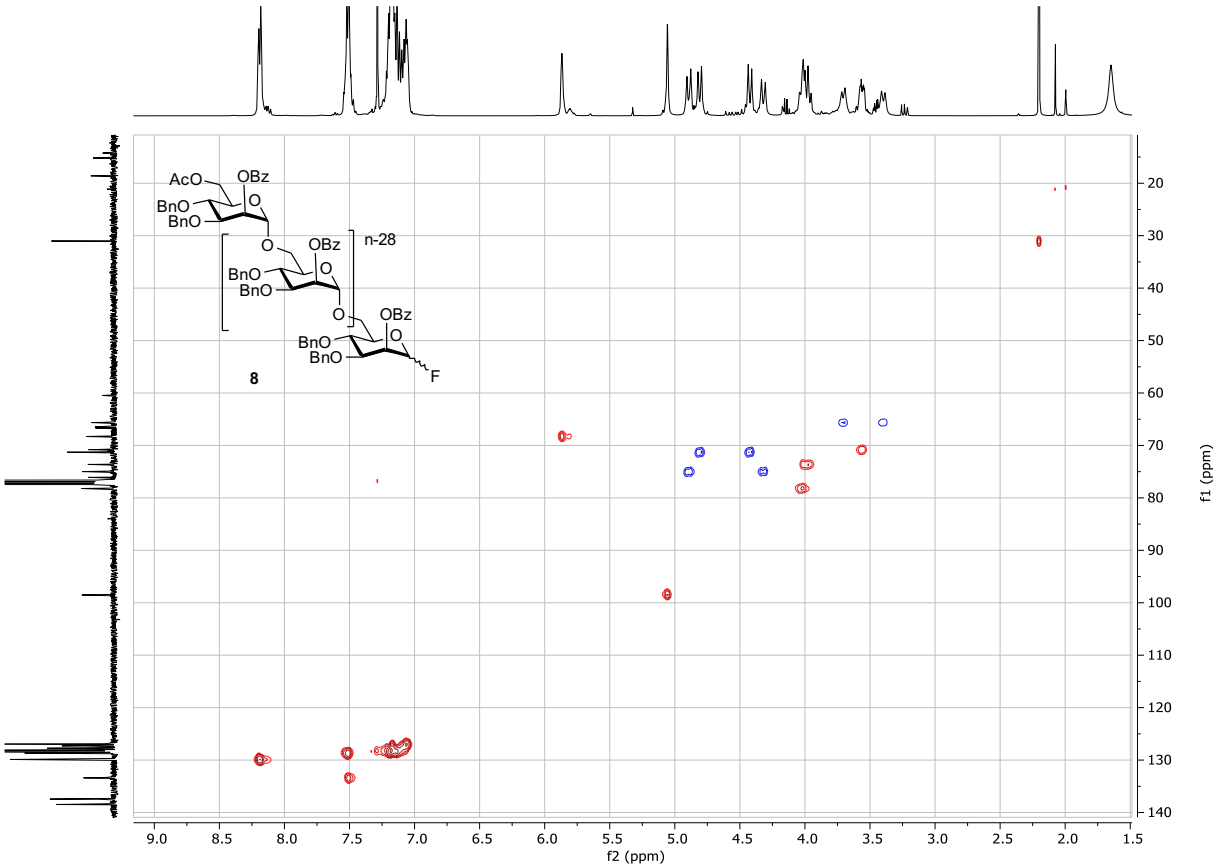
^{1}H NMR of 30-F 30-F donor (**8**)



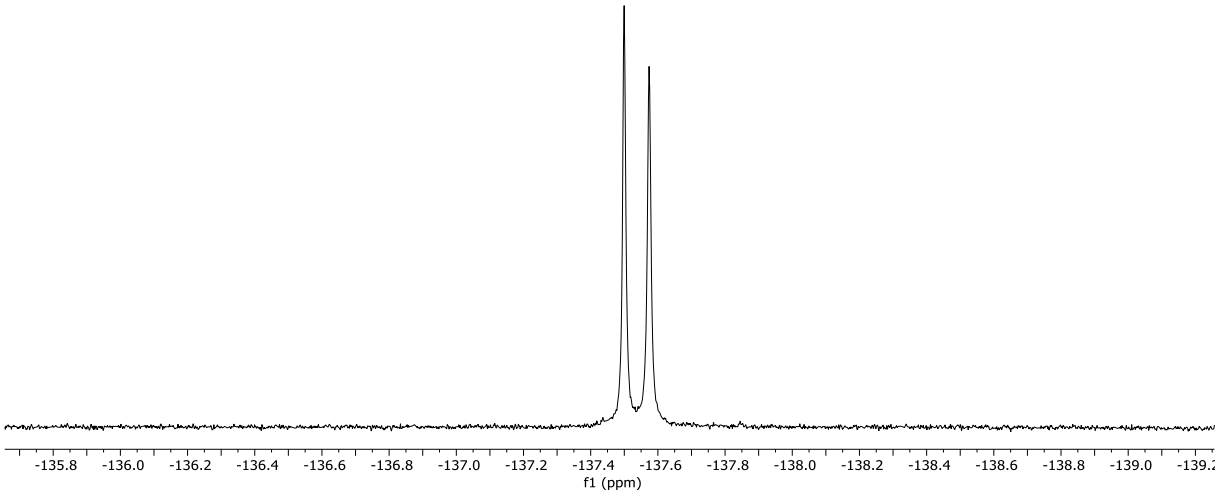
^{13}C NMR of 30-F donor (**8**)



HSQC NMR of 30-F donor (**8**)

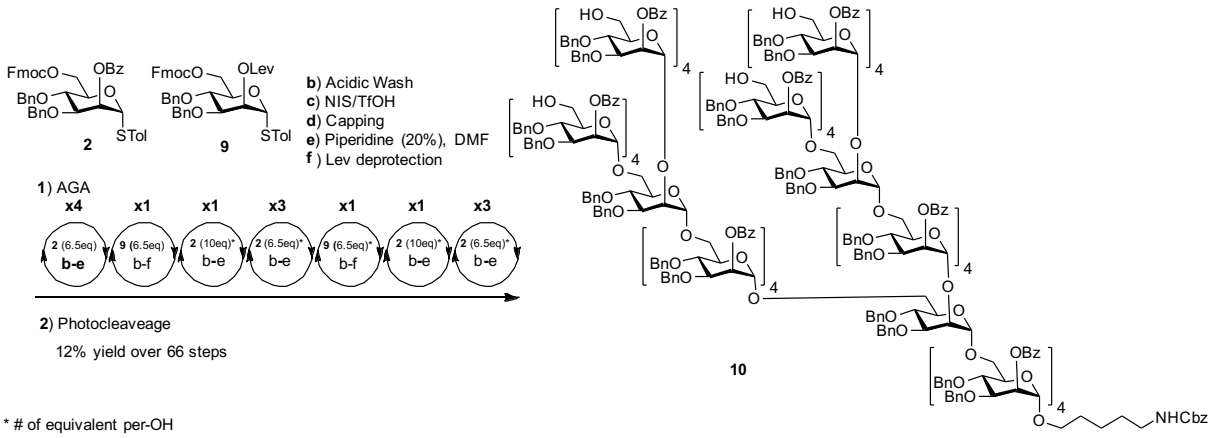


^{19}F NMR of 30-F donor (**8**)



Analytical data for **8**: ^{1}H NMR (700 MHz, Chloroform-*d*) δ 8.21 – 8.17 (m, 60H), 7.51 (qd, *J* = 8.0, 4.2 Hz, 90H), 7.21 – 7.08 (m, 300H), 5.85 (t, *J* = 2.4 Hz, 30H), 5.06 (d, *J* = 1.9 Hz, 30H), 4.89 (d, *J* = 11.5 Hz, 30H), 4.80 (d, *J* = 11.0 Hz, 30H), 4.43 (d, *J* = 11.0 Hz, 30H), 4.35 (dd, *J* = 11.6, 3.4 Hz, 30H), 4.03 (dd, *J* = 9.2, 3.2 Hz, 30H), 4.01 – 3.96 (m, 30H), 3.74 (d, *J* = 11.1 Hz, 30H), 3.61 – 3.57 (m, 30H), 3.45 (dd, *J* = 10.4, 7.7 Hz, 30H), 2.20 (s, OAc, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.54, 138.47, 137.44, 133.39, 129.94, 129.89, 128.68, 128.44, 128.38, 128.14, 127.74, 127.29, 126.94, 98.51, 78.21, 77.26, 76.09, 74.99, 73.63, 71.29, 70.80, 68.29, 65.64, 31.02, 18.59; m/z (MALDI-TOF) 13448.270 $[M + H]^+$ ($C_{812}H_{783}FO_{181}$ requires 13447.2049).

Automated Synthesis of Branched Acceptor 10

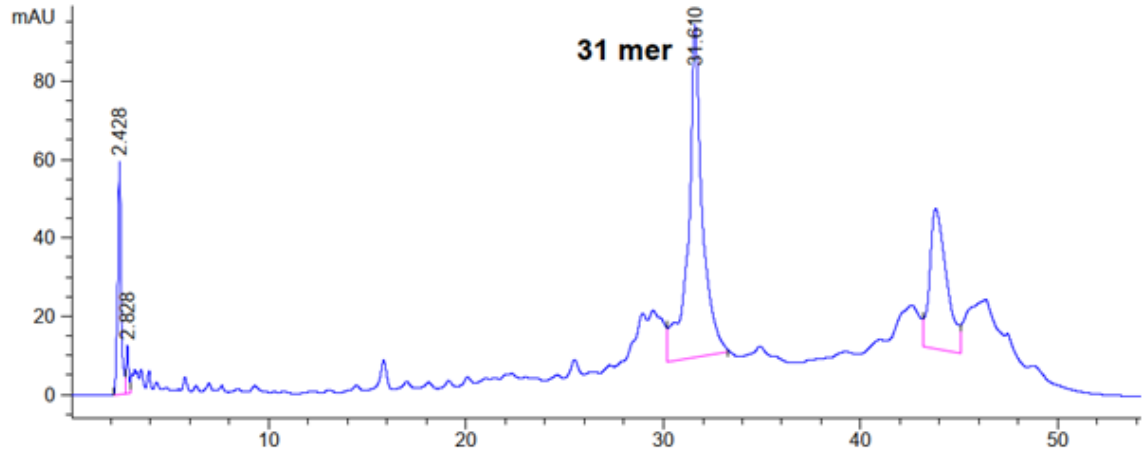


Module	Conditions	Cycles
A: Resin Preparation for Synthesis		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (6.5 equiv)	
	-20° for 5 min, 0° for 20 min	4
D: Capping		
E: Fmoc Deprotection		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **9** (6.5 equiv)	
	-20° for 5 min, 0° for 20 min	1
D: Capping		
F: Lev Deprotection		
E: Fmoc Deprotection		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (10 equiv)	
	-20° for 5 min, 0° for 20 min	1
	Building block **2** (6.5 equiv)	
	20° for 5 min, 0° for 20 min	3
D: Capping		
E: Fmoc Deprotection		

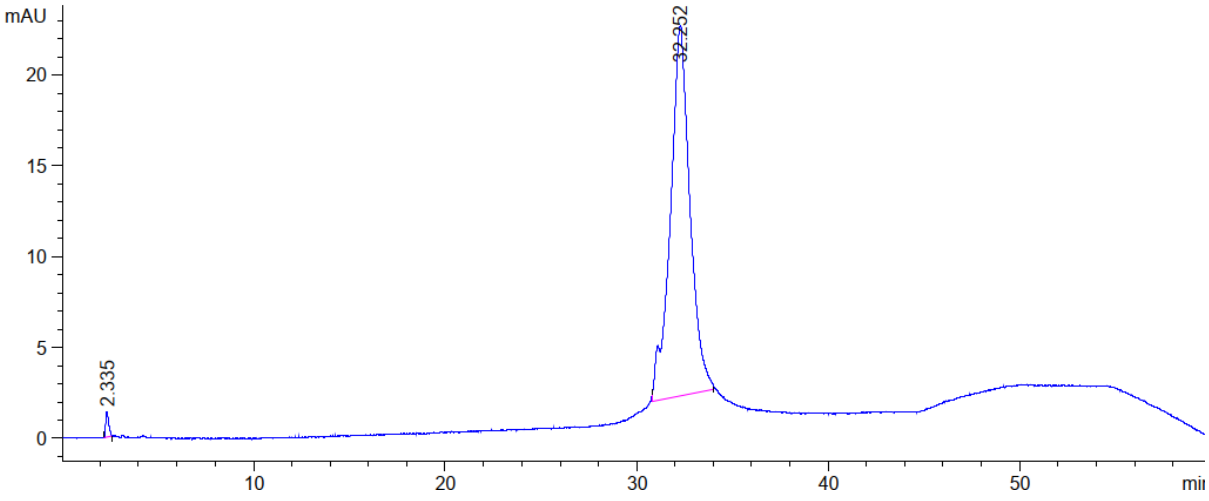
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **9** (6.5equiv) -20^{o} for 5 min, 0^{o} for 20 min	1
D: Capping		
F: Lev Deprotection		
E: Fmoc Deprotection		
B: Acidic Wash with TMSOTf Solution		
C: Thioglycoside Glycosylation	Building block **2** (10 equiv) -20^{o} for 5 min, 0^{o} for 20 min	1
	Building block **2** (6.5 equiv) 20^{o} for 5 min, 0^{o} for 20 min	3
D: Capping		
E: Fmoc Deprotection		
G: Photo-cleavage		

The product was cleaved from the solid support as described in the post-synthesizer manipulations followed by purification by using normal phase preparative HPLC with YMC diol column (Method F) Linear gradient: Hex – 35% EtOAc as eluents [isocratic 35% EtOAc (5 min), linear gradient to 35% EtOAc (5 min), linear gradient to 60% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)] 31 mer **10** eluted at 32.25 minutes.

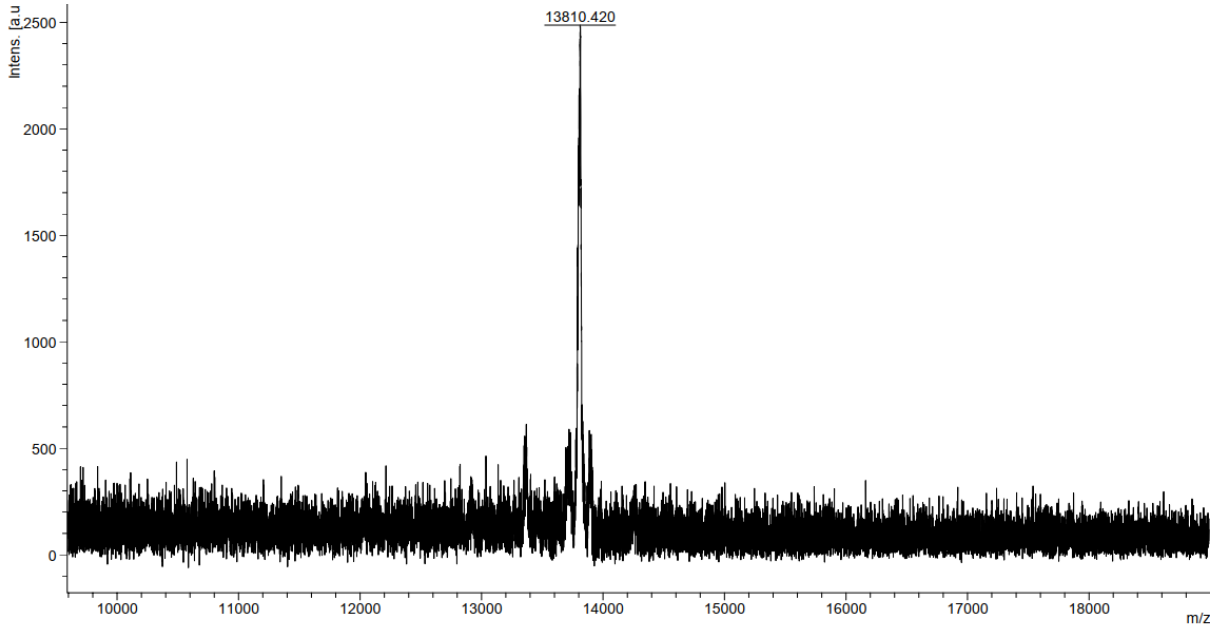
Analytical HPLC (Method E, 280 nm) of 31-mer crude acceptor (**10**)



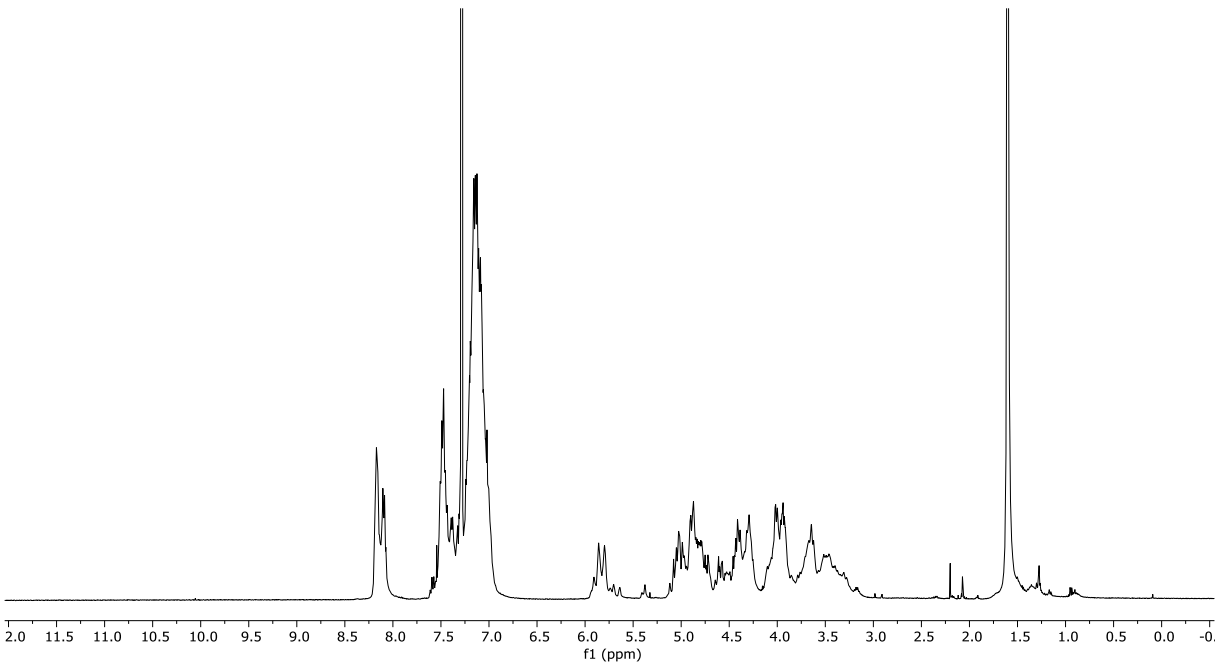
Analytical HPLC (Method E, 280 nm) of 31-mer pure acceptor (**10**)



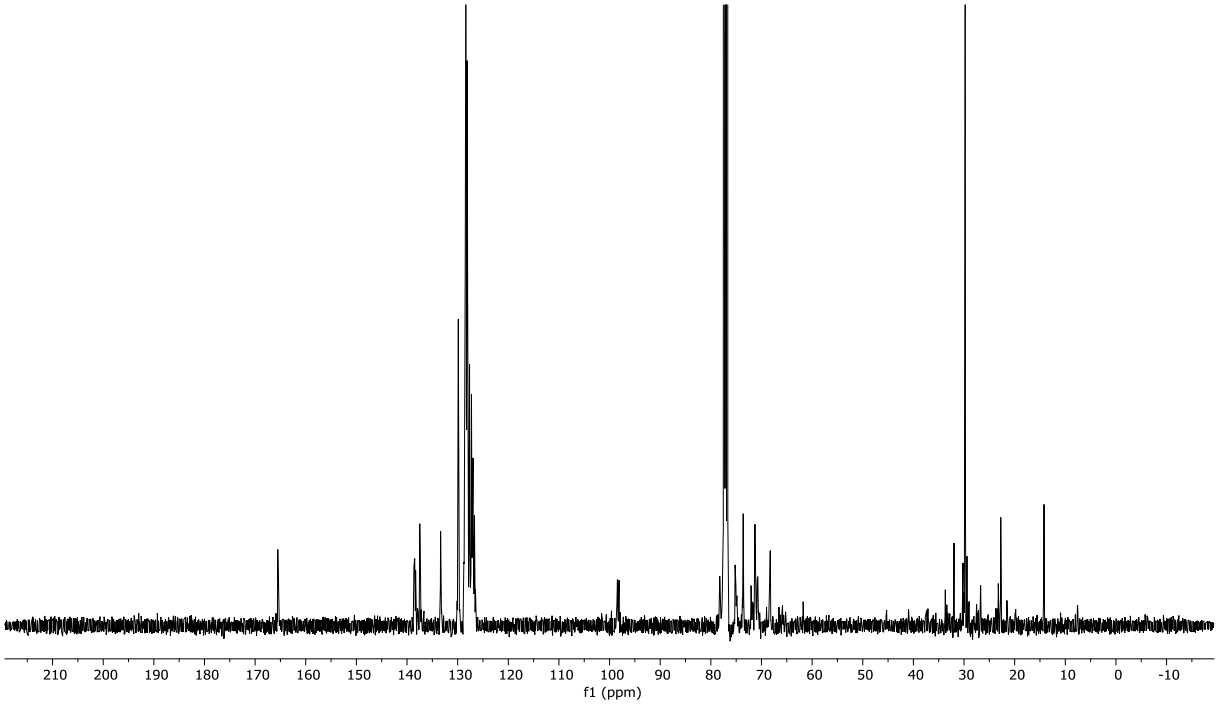
MALDI-TOF of 31-mer acceptor (**10**)



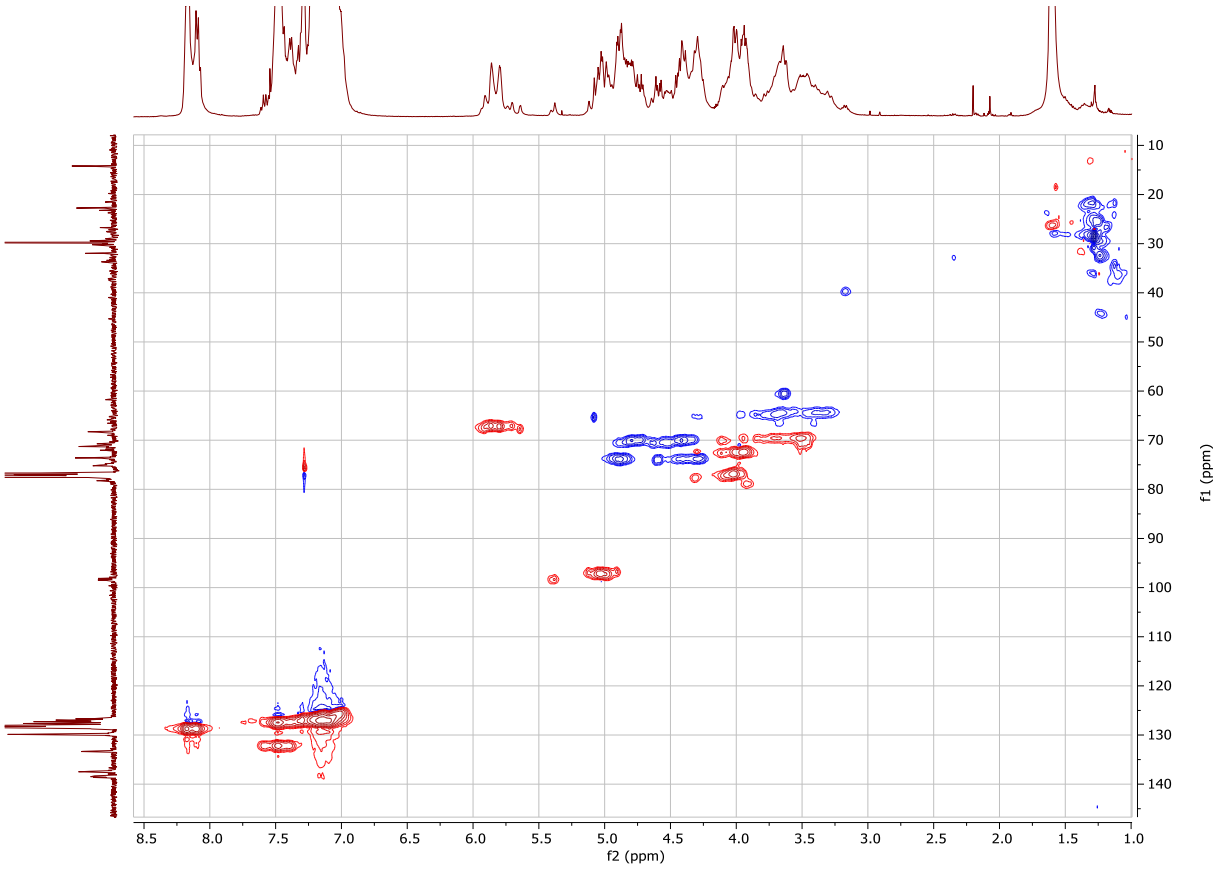
^{1}H NMR of 31-mer acceptor (**10**)



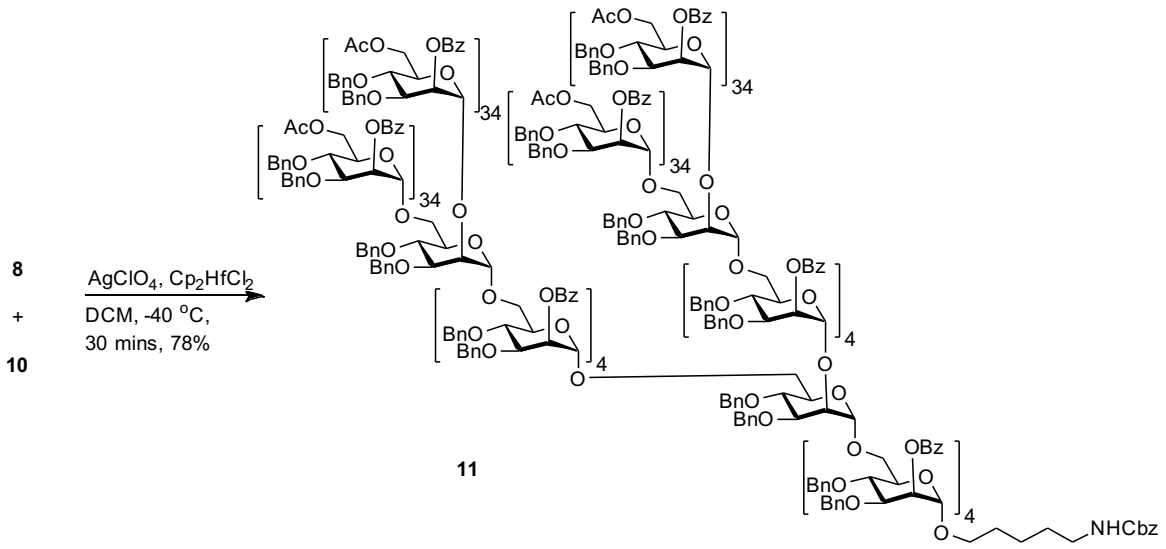
^{13}C NMR of 31-mer acceptor (**10**)



HSQC NMR of 31-mer acceptor (**10**)



Synthesis of Branched 151-mer Polymannoside 11 via Block Coupling



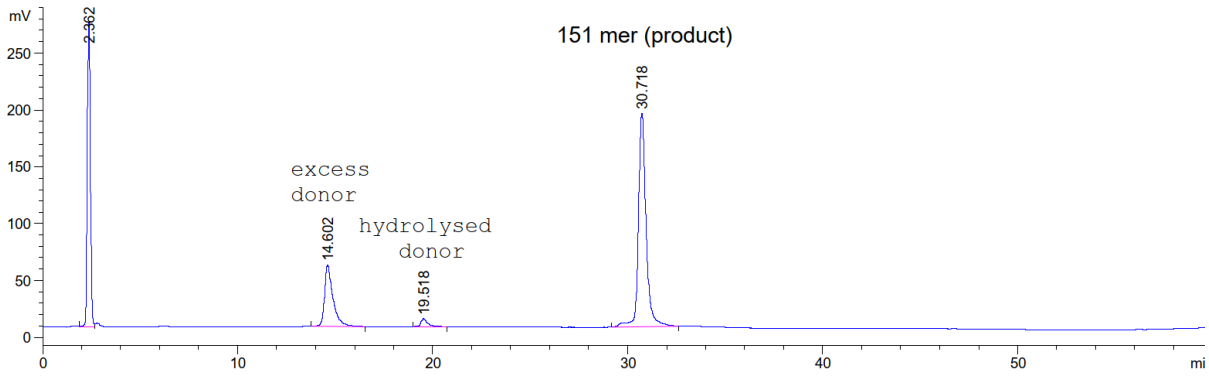
To a solution of glycosyl donor **8** (41 mg, 6 eq) and glycosyl acceptor **10** (7 mg, 1 eq) in 1mL of anhydrous CH_2Cl_2 was added activated 4Å molecular sieves (20 mg) at room temperature for 5 min protected from light. The reaction was cooled to -40 °C and silver perchlorate (6.2 mg, 10 eq) (WARNING: dry perchlorates are explosive!!) and bis(cyclopentadienyl) hafnium dichloride (11 mg, 10 eq) were added.[5] This mixture was allowed to stir at same temperature for 30 minutes to complete the reaction. This mixture was quenched with Et_3N diluted with CH_2Cl_2 and filtered through Celite. The filtrate was extracted with (10 mL) aqueous $NaHCO_3$ and (10 mL) brine. The organic layer was dried over $MgSO_4$ filtered, concentrated and purified by preparative HPLC (Method H) with a YMC diol column. Pure compound **11** was isolated 27 mg in 78% yield.

Optimization of Glycosylation Reaction

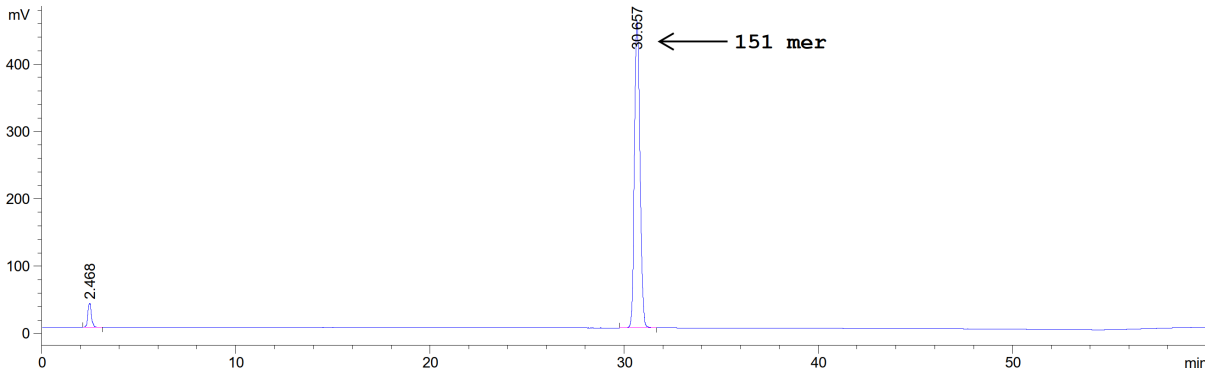
Entry[a]	Leaving group	Promotor	Temperature (°C)	Time (h)	Yield (%)
1	Trichloroacetimidate	TMSOTf	-40 to -5	7	0
2	Fluoride	$AgOTf/Cp_2HfCl_2$	-40 to rt	24	63
3	Fluoride	$AgClO_4/Cp_2HfCl_2$	-40	0.5	78

[a] Reactions conditions: **10** (1 equiv.), **8** (6 equiv.), CH_2Cl_2.

Analytical HPLC (Method G, ELSD trace) of crude 151-mer (**11**)

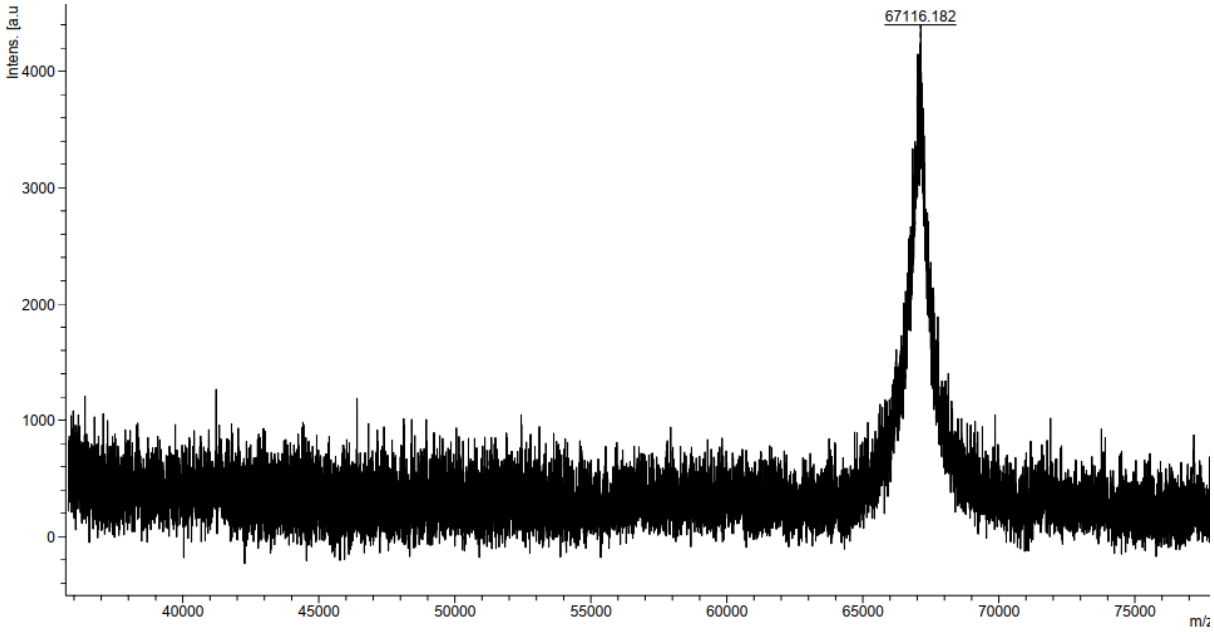


Analytical HPLC (Method G, ELSD trace) of pure 151-mer (**11**)

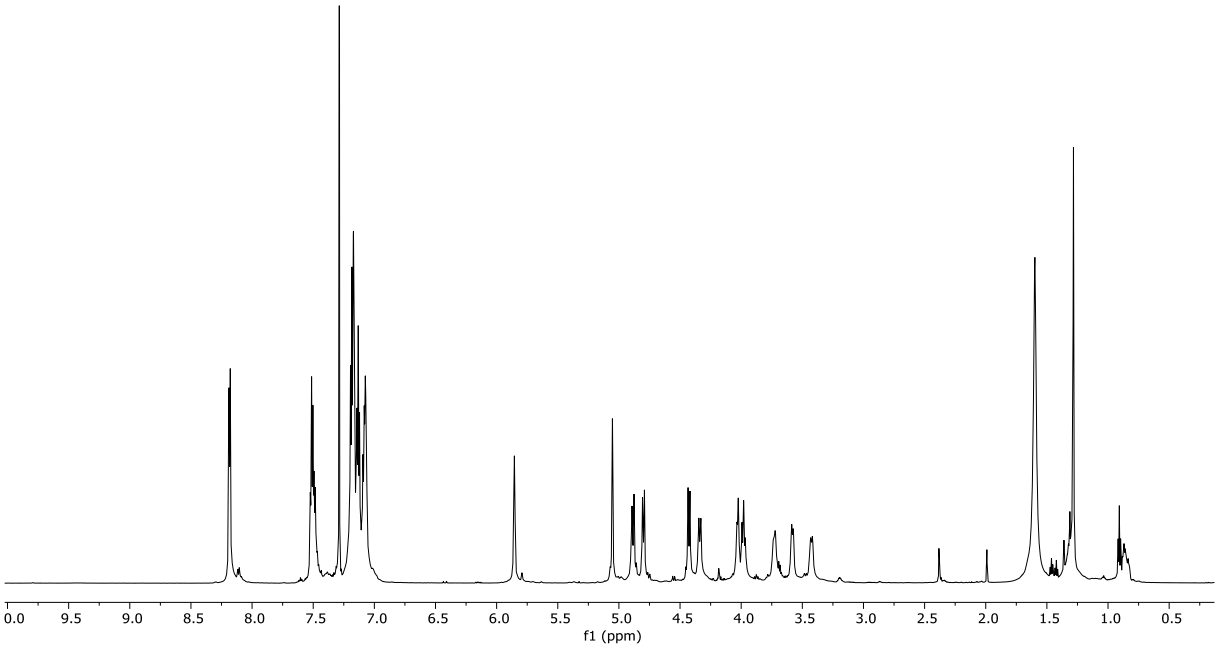


Analytical data for **11**: ^{1}H NMR (700 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 7.6 Hz, 302H), 7.50 (dt, *J* = 13.3, 7.2 Hz, 453H), 7.23 – 7.04 (m, 1510H), 5.85 (d, *J* = 3.2 Hz, 151H), 5.05 (s, 151H), 4.88 (d, *J* = 11.6 Hz, 151H), 4.80 (d, *J* = 10.9 Hz, 151H), 4.43 (d, *J* = 11.0 Hz, 151H), 4.38 – 4.31 (m, 151H), 4.03 (dd, *J* = 9.3, 3.2 Hz, 151H), 3.98 (t, *J* = 9.5 Hz, 151H), 3.79 – 3.65 (m, 151H), 3.58 (d, *J* = 9.6 Hz, 151H), 3.46 – 3.37 (m, 151H), 2.38 (s, OAc, 12H); ^{13}C NMR (176 MHz, Chloroform-*d*) δ 165.54, 138.49, 137.51, 133.32, 130.01, 129.87, 128.65, 128.36, 128.34, 128.14, 127.69, 127.30, 127.02, 98.56, 78.20, 75.00, 73.72, 71.30, 70.90, 68.39, 65.72, 31.94, 29, 22.70; m/z (MALDI-TOF) 67116.182 $[M + Na]^+$ ($C_{4049}H_{3921}NO_{908}K$ requires 67116.0675).

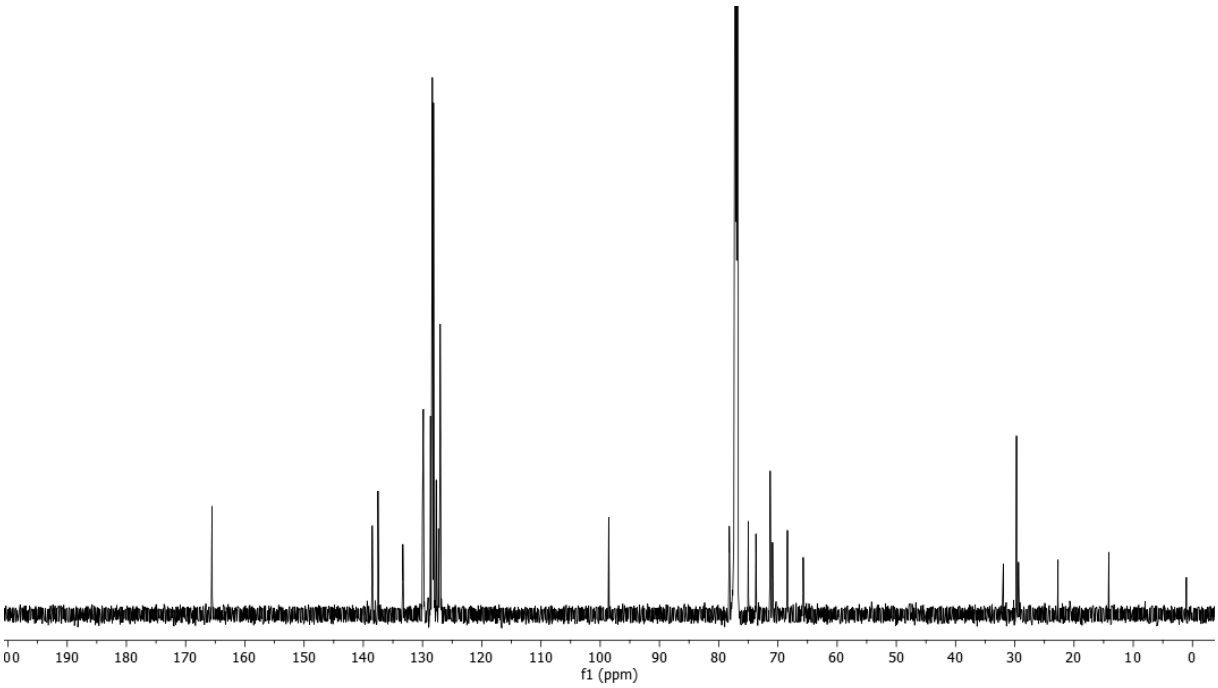
MALDI-TOF of pure 151-mer (**11**)



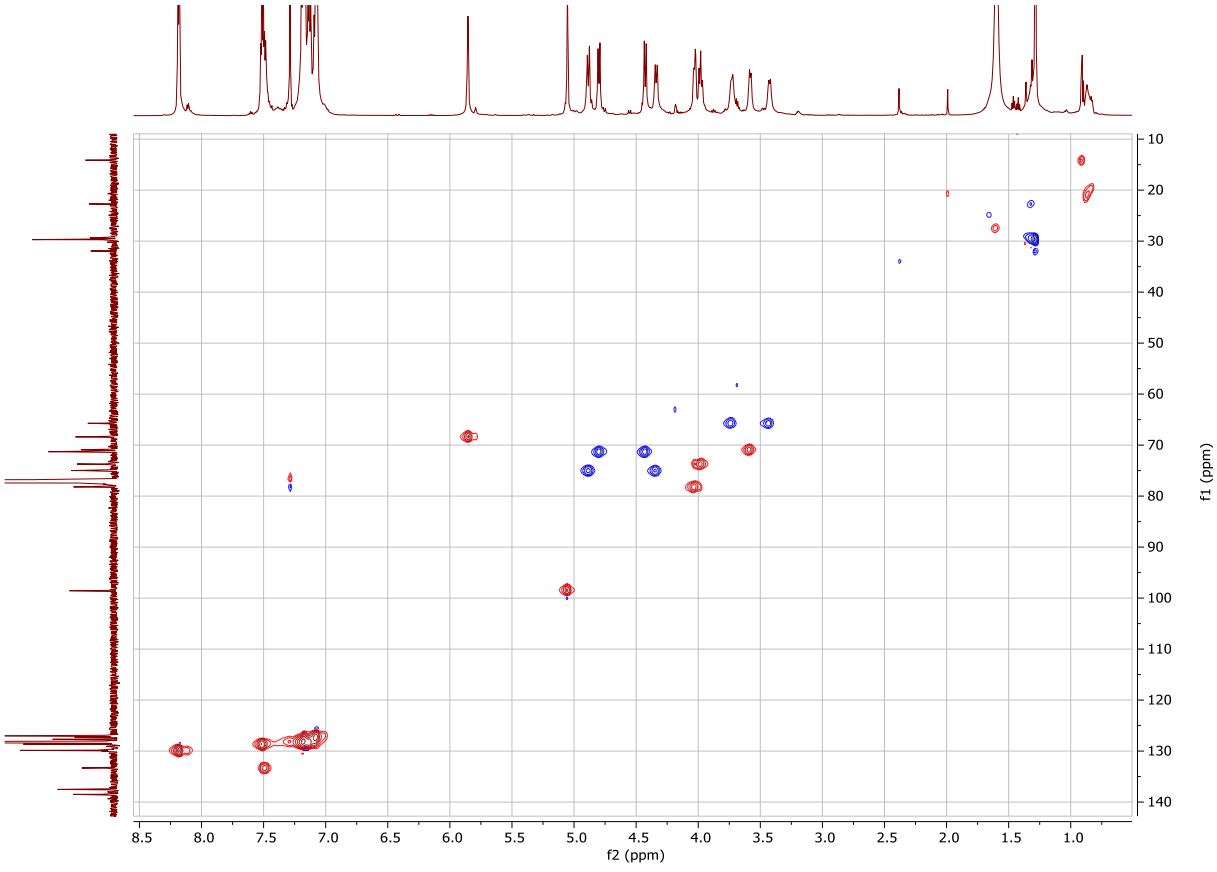
^{1}H NMR of 151-mer (**11**)



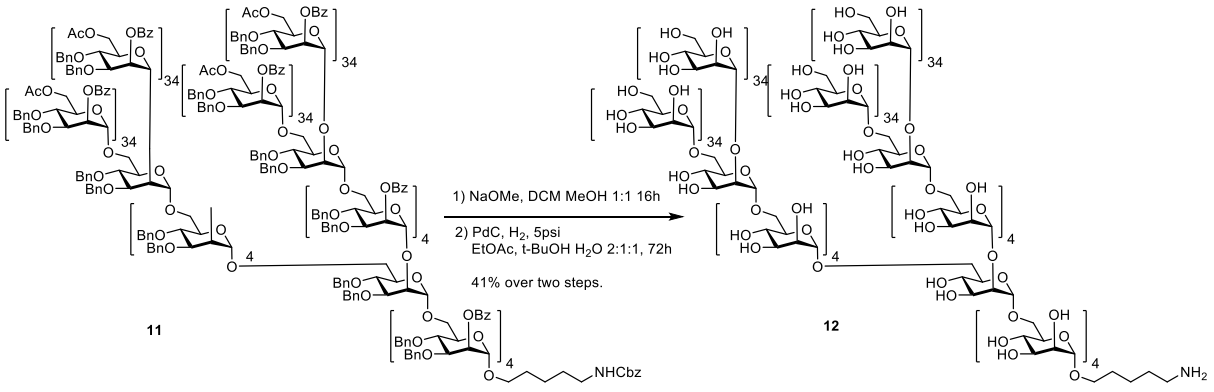
^{13}C NMR of 151-mer (**11**)



HSQC NMR of 151-mer (**11**)



Deprotection of 151-mer polymannoside:

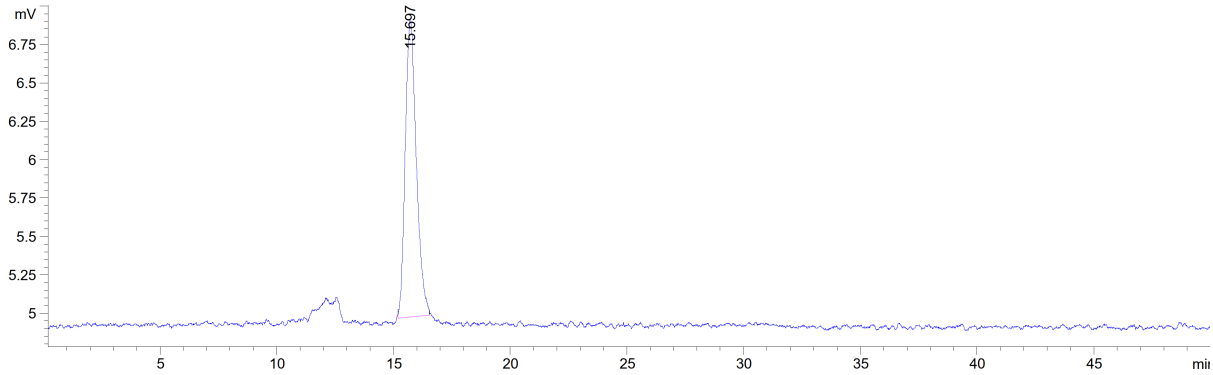


Zemplén Methanolysis. Sodium methoxide in methanol (0.5 M, pH 13) was added to a solution of protected oligosaccharide (12 mg) **11** in methanol:CH_2Cl_2 (1:1), and stirred at room temperature for 16 h, neutralized with Amberlite ion exchange (H+) resin, filtered and concentrated in vacuo and carried forward directly into hydrogenolysis without purification.

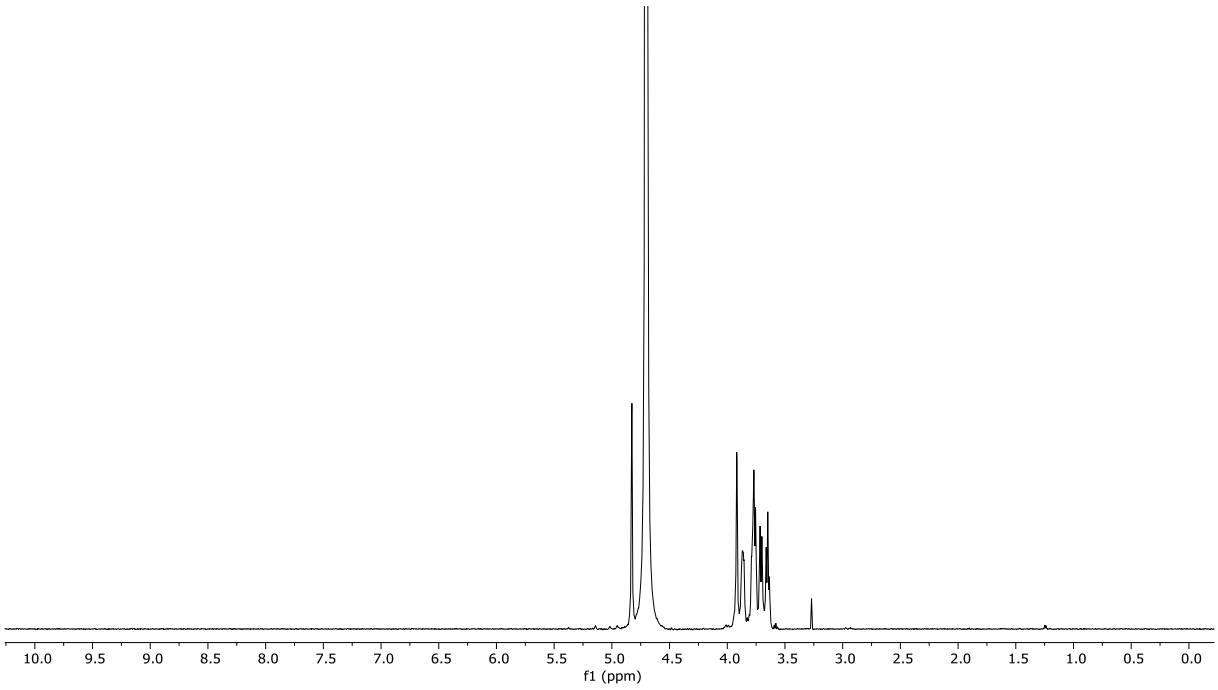
Hydrogenolysis. The Zemplén methanolysis product was dissolved in EtOAc:*t*-BuOH:H_2O (2:1:1) and transferred to cylindrical vials. Pd-C (10%), (100 weight %) was added and the reaction mixture was stirred in hydrogen reactor with 5 psi pressure for 72 h. The reaction mixture were filtered through Celite and washed with methanol and water. The filtrates were concentrated in vacuo and purified on size exclusion chromatography (Method I) TSKgel G3000 PWXL column and lyophilized to give a pure compound **12** in 1.8 mg (41% yield over two steps).

Analytical data for **12**: ^{1}H NMR (700 MHz, D_2O) δ 4.92 (s, 151H), 4.01 (s, 151H), 3.98 – 3.93 (m, 151H), 3.85 (d, *J* = 11.9 Hz, 302H), 3.80 (d, *J* = 11.2 Hz, 151H), 3.74 (t, *J* = 9.9 Hz, 151H), 3.06-3.04 (m, 2H), 3.02 (s, 2H), 2.0 (d, *J* = 6.9 Hz, 2H), 1.33 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (176 MHz, D_2O) δ 99.35, 70.84, 70.70, 69.99, 66.61, 65.52; m/z (MALDI-TOF) 24574.800 $[M+H]^+$ ($C_{911}H_{1524}NO_{756}$ requires 24574.076).

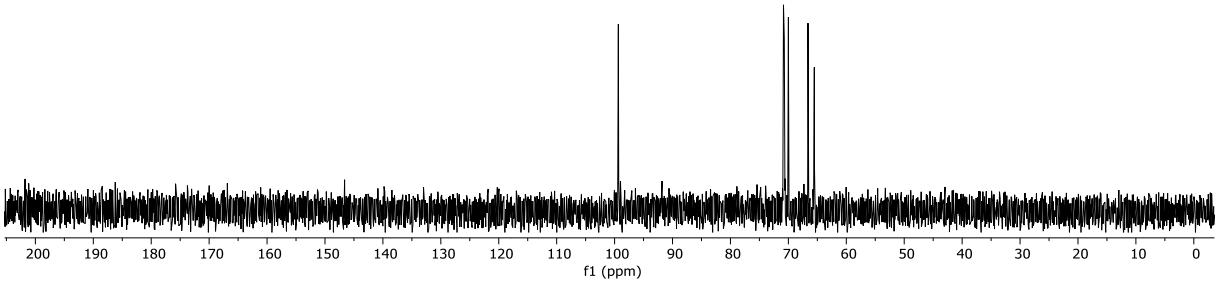
Analytical HPLC (Method I, ELSD trace) of pure 151-mer (**12**)



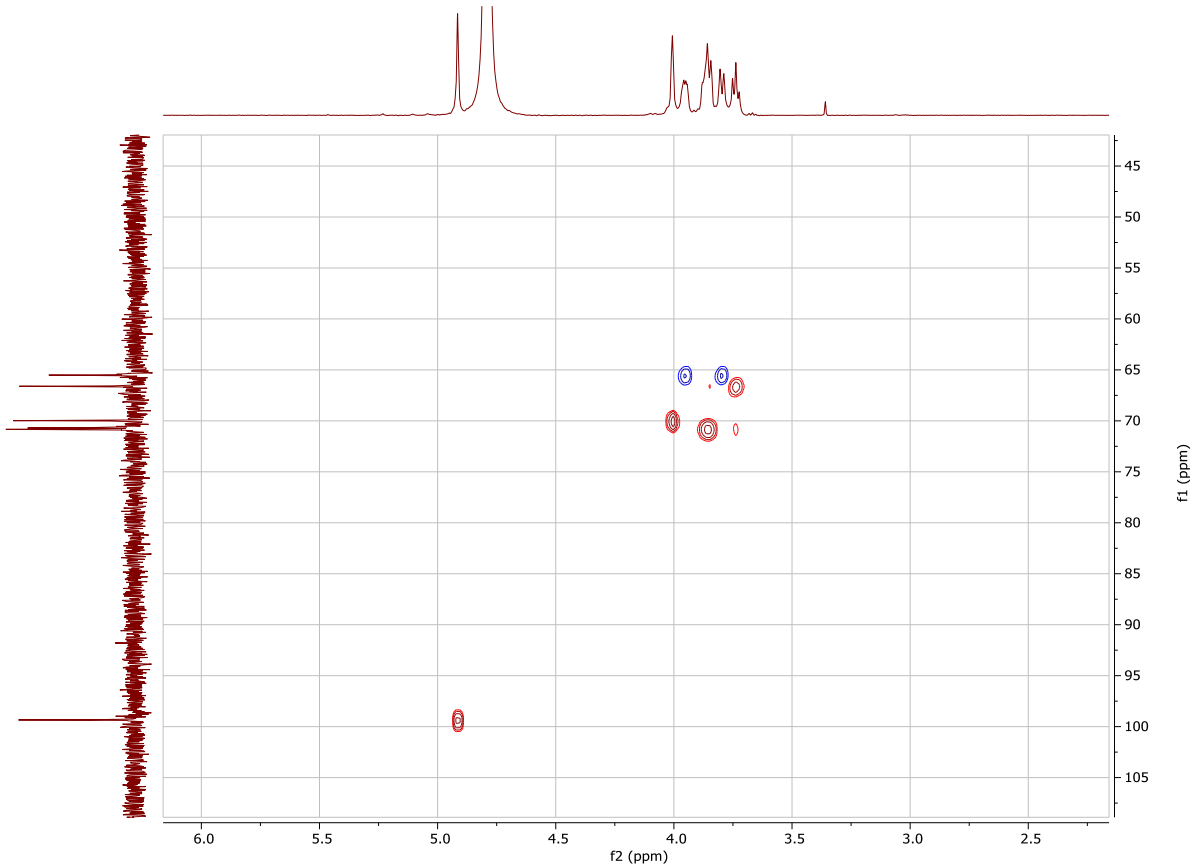
^{1}H NMR of 151-mer (**12**)



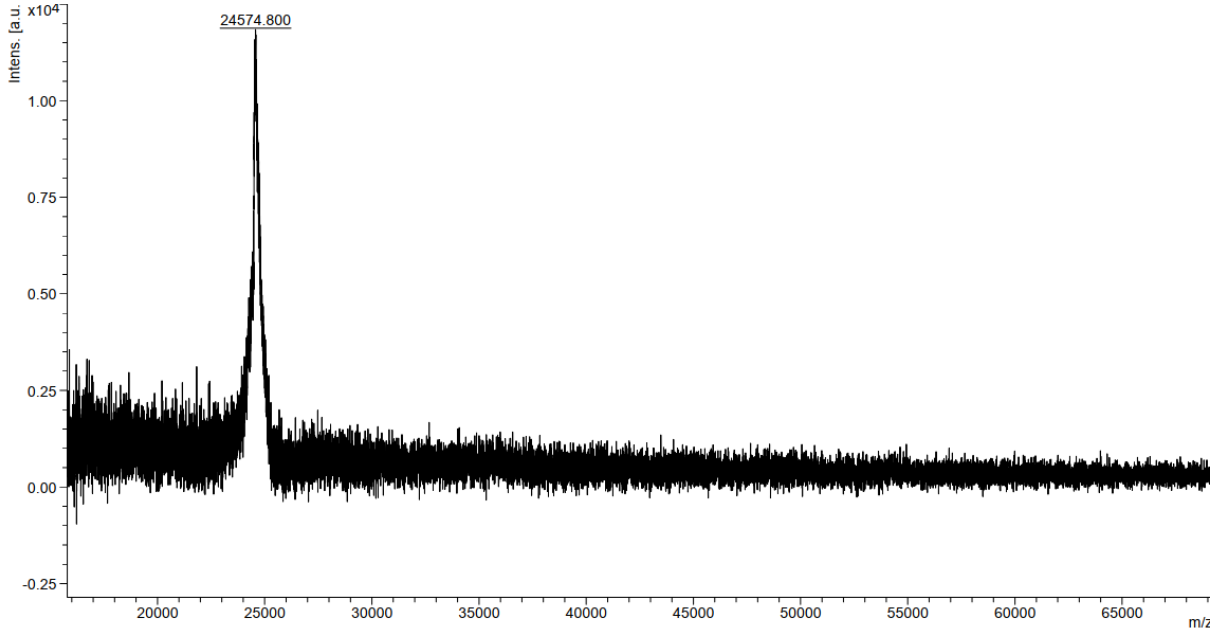
^{13}C NMR of 151-mer (**12**)



HSQC NMR of 151-mer (**12**)



MALDI-TOF of 151-mer (**12**)



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